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The development of liquid and gas chromatographic methods for the determination of water

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The development of liquid and gas chromatographic methods for the determination of water

by

Jian Chen

A Dissertation Submitted to the

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Ames, Iowa

1991

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DEDICATION

This work is dedicated to my wife, Yanwen.

Her unconditional love, support, and encouragement

have made this work a reality.

GENERAL INTRODUCTION

Water is universally present on this planet. Both large- and small-scale processes might be adversely affected by small amounts of water. Consequently, the development of new and/or improved methods for determining water has been the subject of a great many analytical investigations. Although a large number of procedures have been developed for application to specific materials, few of them have proved generally applicable. No single method is applicable to all problems. This is mainly because that the analytical samples involved vary so differently in their physical and chemical properties. The analytical procedure employed will depend on the type of sample matrix, required precision and accuracy, water concentration, as well as equipment available and expertise of the personnel. A comprehensive review on the methods for the determination of water has been written by Smith and Mitchell (1-3). These methods include chemical methods, gravimetric methods, thermal methods, separation methods, spectroscopic methods, as well as other miscellaneous methods. The development and refinement of these techniques is expected to continue in the future due to the great demand in applications.

Among the numerous techniques developed to date, the Karl Fischer

(KF) titration method is by far the most widely used and the most universally applicable method for determining water. This fact is underlined by its incorporation in the most important pharmacopoeias and by its adoption as an ASTM method. A significant amount of investigation and improvement has been made on this method since the first report by Karl Fischer in 1935 (4).

Although the improved Karl Fischer method has been very successful in most applications, it still has some drawbacks. The Karl Fischer reagent itself has a relatively short shelf life due to side reactions and has to be standardized frequently. Side reactions during the titration may also occur since the reaction rate of water and the KF reagent is not very fast. Certain compounds or classes of compound react with the KF reagent, causing serious interference. The sample size required for the KF titration is also relatively large, especially for samples containing small amount of water.

Chromatographic methods offer several attractive features unmatched by the classical titrimetric methods. Since separation is usually completed before the detection, interferences from the sample matrix are eliminated. These methods are often very simple, fast, and quite sensitive. The cost of operation is very low and only small sample size in the order of microliter is required. Most research and quality

control laboratories are now equipped with at least one HPLC or GC chromatograph. It is our goal of study to develop practical methods based on the chromatographic separations which should be easily adopted in those laboratories.

Explanation of Dissertation Format

This dissertation is divided into three sections, each of which represents a distinct method concerning the determination of water. The work described in Section I and II is an extension and refinement of the work initiated by Fortier and Fritz (5). Work in Section I involves significant improvement on a single-column LC method proposed in the earlier work (5). A theoretical equation for the unique spectrophotometric detection system is derived and verified by various experiments. Experimental conditions are systematically studied and optimized so that water can be determined quickly and accurately. The separation mechanism and causes for injection peaks are also addressed. Work in Section II is aimed at the difficult samples encountered by the single-column method. By using a two-column approach, samples such as aldehydes, ketones, and peroxides, which cannot be analyzed by the single-column or Karl Fischer method, are easily analyzed. With this two-column method, no major interference was encountered.

Section III represents a GC method using a similiar indirect detection scheme employed by Dix and Fritz (6). A new reagent and an acid catalyst were found to give faster and more complete reaction than those used in the previous study (6). The analytical procedure was modified so that faster separation, higher sensitivity, and lower limit of detection were achieved.

Sections I through III represent papers in their final publication form with only minor modifications. The introduction in Section I is expended to provide the readers with background in some of the common methods for the determination of water. Portions of the introductions and experimental sections are redundant because each section is complete by itself. Reference to tables, figures, and literatures apply only to those references contained within that section. The literature cited in the General Introduction and General Summary is listed in the General Reference list at the end of the dissertation.

All of the work presented in this dissertation is done under the guidance of Dr. J. S. Fritz and performed at Ames Laboratory, operated by the Chemistry Department, Iowa State University for the U.S. Department of Energy under contract No. W-7405-ENG-82.

SECTION I. SINGLE-COLUMN LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF WATER

INTRODUCTION

Karl Fischer Titration Method

For many years, scientists have shown a lively interest in methods for determining the amount of water in chemical substances. Scores of analytical methods have been devised, ranging from simply weighing a sample before and after drying in an oven to the famous Karl Fischer (KF) titration method. Although a large number of approaches has been used for the determination of water in various analytical samples, the Karl Fisher titration method continues to dominate the field. A great amount of work has been devoted to the study and improvement of the Karl Fischer method since the first report by Fischer in 1935 (1). In the well-known book Aquametry (2-4), one entire volume (4) is dedicated to the determination of water in various samples using the Karl Fischer titration.

Karl Fischer reagent

All of the Karl Fischer procedures are based on the reaction between water and the Karl Fischer reagent (KFR). Although a few modifications have been suggested, the most useful KF reagent is still the conventional or methanol based reagent. It consists of iodine, sulfur

dioxide, pyridine, and methanol with a required minimum ratio of 1:1:3:1 of these components. The methanol serves as the proton donor of the reaction as well as the solvent for the amine salt formed. The composition of a typical KF reagent (4) is given in Table I. The resulting reagent has a water equivalent of about 3.5 mg/ml. Commercial KF reagents are also available from various vendors.

Before Fischer discovered his reagent, it had been well established that at room temperature iodine, like bromine and chloride, reacts with water in the presence of sulfur dioxide according to the reaction:

$$I_2 + SO_2 + 2\,H_2O \longrightarrow 2\,HI + H_2SO_4 \qquad (1)$$

However, the reverse reaction begins to occur when the concentration of the acid is increased to a certain level:

$$H_2SO_4 + 2\,HI \longrightarrow I_2 + H_2SO_3 + H_2O \qquad (2)$$

In his attempts to prevent the reversal of the reaction, Fischer found that it was necessary either to decompose the acid products or introduce some material which would combine with them. The latter method appeared to be the more desirable. A study of the weak amines revealed that pyridine was particularly well suited to this purpose. It was found to have additional advantage of combination with the sulfur

Table I. Composition of a typical Karl Fischer reagent

Substance	Quantity per liter of reagent	Total quantity
Iodine	84.7 g	762 g
Sulfur dioxide	45 ml (64 g)	135 ml
Pyridine	249 ml	2420 ml
Methanol	667 ml	6000 ml

dioxide, thereby reducing the latter's vapor pressure. A tertiary amine thus became an essential component of the reagent.

A thorough study (5) of the stoichiometry of the reaction between water and the Karl Fischer reagent indicates that the main reaction in the methanol solution appears to take place in two distinct steps as follows:

$$I_2 \cdot Pyr + SO_2 \cdot Pyr + Pyr + H_2O \longrightarrow 2\ PyrH^+I^- + SO_3 \cdot Pyr \qquad (3)$$

and

$$SO_3 \cdot Pyr + CH_3OH \longrightarrow PyrH^+CH_3SO_4^- \qquad (4)$$

This proposed reaction mechanism is supported by the fact that actually

only 1 mole or less water is removed by 1 mole of I_2 in the reagent.

While Equation 3 and 4 predicts the maximum absorption of water by the regular reagent as 1 mole per mole of iodine, this theoretical efficiency is rarely attained because of side reactions. Including the correction of the water in the components, the freshly prepared reagent usually is equivalent to about 80% of the theoretical strength, but in the course of about a month, its strength falls to about 50% of the theoretical value (4).

The side reactions for the methanol reagent have been found to include the reduction of iodine to the iodide ion and the formation of quaternary methylpyridium salts (5):

$$I_2 + SO_2 + 3\ Pyr + 2\ CH_3OH \longrightarrow PyrCH_3^+SO_4CH_3^- + 2\ PyrH^+I^- \qquad (5)$$

$$I_2 + 2\ SO_2 + 4\ Pyr + 3\ CH_3OH \longrightarrow PyrH^+SO_4CH_3^- + PyrCH_3^+SO_4CH_3^- + 2\ PyrH^+I^- \qquad (6)$$

Also the types of impurities in the pyridine affect the rate of the degradation of Fischer reagent (6,7).

One way to prevent the side reactions is to not add sulfur dioxide until shortly before use. Two solutions were prepared for this purpose (8). One contained methanol, pyridine and sulfur dioxide and the other,

iodine dissolved in methanol. Consequently, active reagent was formed only during the actual titration, and where the rate of reaction of water was considerably greater than other iodine-consuming reactions, little interference was observed.

Other modifications have included the use of certain salts to reduce rates of side reactions, substitutes for compounds used in conventional reagent, and variations in titration techniques (9-14).

Karl Fischer titration

Several key steps of an actual KF titration include sample preparation, standardization of the reagent, and determination of the endpoint.

Analyses for water in liquids are usually straight forward. Methanol and pyridine provide desirable miscibility with the sample and solubility of Karl Fischer reagent end products. Although homogeneous solutions are most desirable for titrations, in many cases successful liquid-liquid extractions have been made directly in the flasks simply by stirring the two-phase system during titrations.

Inert solids that are soluble in an inert liquid suitable for KF titrations (e.g., methanol, pyridine, glycol, and dimethylformamide) can be titrated directly. In these cases, total water (i.e., free plus

combined) is determined. Where the solid sample is finely divided, it usually can be titrated as a dispersion. In this situation, only free water is titrated.

As discussed earlier, the Fischer reagent can be expected to decease in strength due to side reactions and absorption of ambient moisture. For this reason, at least one standardization a day must be performed for most routine work. For maximum accuracy, however, the water equivalence of the reagent should be checked with each set of samples. The reagent may be standardized conveniently by titration of weighed quantities of stable salt hydrates, of measured amounts of water, or of precisely measured volumes of methanol containing a known amount of water.

Perhaps the most important step in a KF titration is to determine the endpoint. Numerous studies have been focused on this subject. So far, four different approaches have been successfully applied. These are visual endpoint procedure, dead-stop (biamperometric) and potentiometric techniques, and coulometric titration method.

The visual endpoint procedure, which requires only simple apparatus and permits rapid titrations, has been the earliest and simplest method for water analyses in general. This approach calls for the titration of a colorless sample solution by KF reagent to the first appearance of

excess iodine, which is indicated by the color change of chromate yellow to the red-brown of iodine. The endpoint is sharp, reproducible, and can be mastered by little practice. With this approach, samples containing 50 to 250 mg of water can be easily analyzed with a precision of 0.2%.

The electrometric methods, on the other hand, are more broadly applicable. They are capable of considerably lower limits of detection, and are more sensitive than the visual method. They are usually the methods of choice when deeply colored solutions are encountered.

The dead-stop endpoint detection (15) depends on the fact that when an electromotive force of 10 to 15 millivolts is impressed upon two platinum electrodes immersed in the Fischer reagent, sufficient current flows through the solution to deflect a galvanometer off the scale. During the titration of the Fischer reagent with a standard solution of water in methanol, the galvanometer remains deflected until the endpoint is approached. The reverse titration, i.e., the addition of Fischer reagent to a solution containing water, was found to be less satisfactory. As a result, the indirect titration, i.e., the back titration of the excess Fischer reagent added to an unknown sample by a water standard is preferred. In the potentiometric approach (16), a constant small polarizing current (e.g., 10 μA) is maintained during the

titration while the potential difference between the two platinum electrodes is monitored. A sudden increase in the potential difference indicates the endpoint.

In a coulometric titration (17,18), iodine needed in the reaction is electrochemically generated in situ during the titration as follows:

$$2\ PyrI^{-}H^{+} + Pyr \xrightarrow{-\ 2e} Pyr{\cdot}I_2 + 2\ PyrH^{+} \qquad (7)$$

The advantages of this approach include less need to prepare and store the highly reactive complete KFR and likely reduction in side or interfering reactions, where the rate of reaction of KFR with water is significantly faster than that of interfering species.

For most applications the "dead-stop" and potentiometric titration methods are equally useful; the technique of choice may depend on the equipment available in the laboratory. The "dead-stop" method is the simplest to use and requires less time than the other electrometric methods. The potentiometric method tends to be more precise. Highest sensitivity is provided by the coulometric procedure. This procedure is particularly valuable for trace analyses of water in small samples (e.g., micrograms of water on milligram samples).

Regardless of the method used, the apparatus must be protected from outside sources of moisture; and the more sensitive the method, the

better the protection must be.

Interferences

A major drawback of the KF titration method is that it is prone to various interferences. Although most substances are inert to KFR, a number of compounds and certain classes of compounds can react with one or more components of KFR. Aldehydes and ketones react to varying extents with methanol in the reagent to form water, resulting in positive error. Others react stoichiometrically with KFR and are listed in Table II.

Methods have been developed to eliminate or correct the interferences. For example, carbonyl compounds may be combined as the cyanohydrins before titrating for water. Isooctene or acrylonitrile is used to combine with mercaptans prior to the analysis. Excess acetic acid is used to eliminate amine and hydrazine interferences. Using different reactions and techniques, most of the interferences can be eliminated or minimized. A complete discussion of this topic can be found in reference (4). Nevertheless, these extra steps complicate the titration procedure and prolong the analysis time. Moreover, they may introduce other interfering factors and reduce the accuracy and precision of KF titration.

Table II. Compounds that react stiochiometrically with Karl Fischer reagent

l-Ascorbic acid	Cupric salts
Hydrazine salts	Ferric salts
Substituted hydrazine salts	Metal hydroxides
Mercaptans	Metal oxides
Hydrogen peroxides	Sodium arsenate
Alkali carbonates	Sodium arsenite
Alkali sulfites	Sodium tetraborate
Alkali pyrosulfites	Sodium thiosulfate
Boric acid and oxides	Stannous chloride

Besides interferences, there are several additional drawbacks associated with the standard KF titration. First it is quite time-consuming because of the rather slow reaction rate of water and KFR near the endpoint of the titration. Second, the titration and detection systems used in the the KF titration are usually dedicated to this purpose only; some of them can be quite costly and are only economically feasible for those routine users. Finally, the analyst has to handle rather large volumes of toxic reagent which is potentially harmful.

In order to over come some of these drawbacks, methods involving other principals and techniques have been developed.

Flow-Injection Analysis with KF Reagent

The introduction of flow-injection analysis (FIA) techniques (19) provides one answer to the increasing load being imposed on the analytical chemists. Applications of this valuable technique have been found in many fields of chemical analysis, including the determination of water with KF reagent (18, 20-25).

Compared to the batchwise Karl Fischer titrations, the FIA method offers several advantages: high sampling rate, over 250 samples per hour; low consumption of the reagent, about 0.5 ml per sample; low sample volumes, 2 - 10 μl; a closed system, which means minimum contact with the toxic reagent; good reproducibility, relative standard deviations are usually less than 2%; no need for calibration of the Karl Fischer reagent; no problems with the humidity of air in the titration vessel; and the ease of automation.

Cedergren and co-workers constructed a special potentiometric detector for the determination of water by FIA with Karl Fischer reagent which showed a relative standard deviation of less than 0.5% (20). A simple potentiometric detection system was reported by Escott and Taylor which gave a linear water concentration range of 0 - 1000 ppm (23). Spectrophotometric detection at 625 nm was found by Cedergren et al. to give a broader water concentration range of 0.01 - 5% (20). The main

disadvantage with the method was the rather large variation between the calibration curves for different types of samples (organic solvents). This solvent effect may have resulted from several factors including viscosity, refractive index, and absorptivity of the sample matrix. Systematic studies on these factors were reported by the same author and his co-workers (21,22). They concluded that the use of a spectrophotometric cell which minimizes the refractive index is necessary for attaining small spreads between the calibration curves for different solvents. The best result were obtained by combining peak area measurements with the use of this detector. Also care must be taken in the choice of the solvent for the standard solutions to keep the matrix effect low.

Another drawback with the FIA method results from the requirement for standards which have to be regularly determined with another method. To overcome the limitations with the FIA method, an alternative method was developed by Liang (25). In his method, an automated sampling system was coupled to the existing coulometric titrators for either on-line analysis or laboratory applications. With this method, the coulometric ability of measuring water over a wide range was preserved while the drawbacks associated with the manual sample introduction were eliminated. The effectiveness of this apparatus as an on-line process

monitor was demonstrated by monitoring the drying of a bottle of wet N-methyl-2-pyrrolidone by molecular sieves.

Gas and Liquid Chromatographic Methods

As discussed above, the Karl Fischer method has certain limitations, such as side reactions of the Fischer reagent, interferences from certain compounds, requirement of large sample volume, modest detection limit of only a few milligrams of water, relatively long analysis time, and incapability for analysis of gaseous samples.

In searching for alternative methods, chromatographic techniques have been employed. There are a number of general advantages associated with the chromatographic methods. First, the separations usually take place before the detection, thus the interference from the sample matrix is eliminated. Second, they can be very fast, sensitive, and low in detection limit, provided that optimized conditions and sensitive detectors are used. Third, only small sample size is required. Last, the operational cost is very low and automation can be easily accomplished. Methods based on either gas or liquid chromatographic techniques have been developed (26-49).

Gas chromatographic method is perhaps the second most popular method for determining water in various samples. Water can be determined

either directly after separation by gas chromatography using a universal detector such as thermal conductivity detector (TCD) or indirectly after chemical conversion. In the later approach, one of the product generated by the reaction is subsequently separated by gas chromatography and determined by a more sensitive detector such as flame ionization detector (FID).

A number of methods have been reported on separation of water on a packed GC column in conjunction with a TCD (26-28). Determinations of 0.03-2 percent water in dimethylformamide and acetone have been made at 100 °C through columns packed with Porapak Q and Synachrom E5 (divinylbenzene-styrene copolymer). A maximum error of 0.07 percent in two parallel determinations is reported by Korarik (26). Water in lyophilized pharmaceutical products is determined by dissolving in dry ethanol and separation on a Porapak QS column at 110 °C, using a TCD and methanol as the internal standard (27). The lowest water reported was 0.1%. Sakano and co-workers (28) developed a relatively simple, rapid GC method for water in chlorinated organic solvents containing active chlorine and hydrogen chloride. The water peak is distinctly and sharply separated from other peaks on a column packed with Porapak Q at a oven temperature of 130 °C. Results on samples of carbon tetrachloride, chloroform, dichloromethane, and chloroethane containing

from 20 to 1000 ppm water and up to 0.6 percent chlorine with 0.2 percent hydrogen chloride agreed within 10 percent between the gas chromatographic and KFR methods. As little as 2 ppm of water could be detected.

One major drawback of the thermal conductivity detector, besides its very large cell volume and modest sensitivity, is the incompatibility with capillary GC columns which are now extensively used in all laboratories. To overcome this problem, a helium ionization detector (HID) which is also a universal detector, was employed by Andrawes (29,30). This sensitive detector is compatible with capillary GC columns. Concentrations as low as 2 ppm of water were detected. Various gaseous as well as liquid samples have be successfully analyzed. Unfortunately, this detection system is linear only up to 700 ppm water. Also the popularity of the HID is currently held back by some difficulties in its operation. Kolb and Auer (31,32) reported an equilibrium headspace gas chromatography (HSGC) method for determining water in liquid and solid samples using a hot wire detector (HWD) in conjunction with a capillary GC column. According to the authors, the water blank resulted from an empty sample vial appeared to be the limiting factor for the detection limit, not the detector. Standard addition was used as the preferred method for quantitation. The limit

of detection was reported as 50 ppm water.

A major limitation of the GC methods discussed above is the lack of using a standard detector. Approaches based on the chemical conversion take advantage of the attractive features offered by FID. This detector is not only sensitive, but also has a broad linear range of up to 8 orders of magnitude. It responses to almost all of the organic compounds and has become the standard detector for GC analyses.

The reactions of water with sodium (33), lithium aluminum hydride (34), calcium carbide (35-38), or 2,2 dimethoxypropane (DMP) (39-43) have been utilized in determining water by gas chromatography with most work focused on the last two reactions.

Latif and co-workers (36) reported a relatively simple procedure using a calcium carbide flow reactor. Traces of water in nitrogen gas were converted to acetylene by passing through a 1 m x 2 mm i.d. glass column packed with calcium carbide and heated at 60 °C. The generated acetylene was then analyzed by using a 2 m x 2 mm i.d. Porapak P column and FID. It was reported, however, that the equilibration time for the reactor between two determinations is a function of both temperature and flow rate. Optimum conditions must be found for a particular column in order obtain reproducible and accurate results.

Loeper and Grob (37,38) utilized the same reaction in their methods

for determining water using headspace gas chromatography (HSGC) coupled with a FID. Both liquid and gaseous samples have been analyzed with these procedures. However, reasonable reproducibility (less than 5%) was obtained only for concentrations raging from 60 to 400 ppm of water. Besides the poor precision, tedious sample manipulation and long reaction time (18 hours) also make this method impractical.

A few authors have used the acid-catalyzed hydrolysis of DMP as a way to determine water. Critchfield and Bishop (39) determined water by reaction of DMP in the presence of methanesulfonic acid and measured the acetone formed by infrared spectroscopy at 5.75 μm. Hager and Baker (40) made a cursory investigation of the use of DMP for the indirect GC determination of water. Martin and Knevel (41) proposed a quantitative method for water by reaction with DMP and measurement of the change in height of the GC peaks of DMP and acetone. The method required accurate weighing of both DMP and acetone, as well as the sample itself. Blanco et al. (42) used a somewhat similar method for determining water in nitroglycerin-nitrocellulose pastes by GC.

The most recent work in this direction is the one reported by Dix and Fritz (43). In their method the sample is combined with a solution containing DMP and an internal standard. A small amount of Nafion is added to catalyze the reaction of water with DMP. After reaching

completion, an aliquot of the reaction mixture is separated on a capillary GC column and one of the products, acetone or methanol is determined by a FID. The usefulness of this method has been demonstrated with a wide variety of samples. Although this method is sensitive and the total analysis time is only about 10 minutes, there are still a few aspects which need to be improved. First the DMP reagent itself gives incomplete reaction at low water levels owing to the modest equilibrium constant for the reaction. Second, the solid acid catalyst has to be weighed out for each sample, which is time-consuming and labor intensive. Third, due to the heterogeneous nature of the acid catalyst, the reaction mixture must be shaken constantly for at least five minutes in order for the reaction to reach completion. The work presented in Section III of this dissertation is intended to solve these problems.

There have been few reports on the use of liquid chromatography for water determination as compared to the large numbers of GC methods developed. As in gas chromatography, both direct and indirect approaches have been proposed using liquid chromatographic techniques.

Small amounts of water in hydrocarbons were determined by Frehrmann and Schnabel (44) using gel chromatography. With toluene as the eluent, water was strongly retarded and well separated from the sample matrix.

By applying a differential refractometer as detector, water concentrations down to 1 x 10^{-4} M could be determined. The lower limit for qualitative detection of water was about 10^{-5} M.

Bjorkqvist and Toivonen (45) discovered that water could be determined by reaction with phenyl isocyanate to form N,N'-diphenylurea which is very stable and highly UV-absorbing. This reaction product could easily be chromatographed by reverse-phase HPLC. A theoretical detection limit of < 100 pg water was claimed by the authors. However, a relatively small number of data obtained with the method showed poor agreement with that obtained with Karl Fischer method. More importantly, one determination, including the half an hour reaction, would require a total of 45 minutes.

Ion-exclusion chromatography (IEC) has been shown to be a fast and efficient way to separate and determine molecular compounds such as carboxlic acids, carbon dioxide (as carbonic acid) (46) and neutral substances such as alcohols and sugars (47). The determination of water by ion-exclusion chromatography should also be possible provided a suitable detection method is available.

Stevens, Chritz and Small (48) were faced with need to determine water in commercial formulations of dibromonitrilopropionamide (DBNPA). DBNPA is an oxidizing agent and interferes with the KF method. It is

also thermally labile and decomposed on a GC column. The product of decomposition posed a serious problem on the GC detector. A method based on a high performance liquid chromatography (HPLC) system was thus developed. In that system, water is separated from the sample matrix on a cation-exchange column in conjunction with a methanol eluent containing dilute mineral acid and determined by a change in conductance resulted from the presence of water. Although their method is fast and convenient, the sensitivity varies widely in different ranges of water concentration. Therefore, it is possible for two different water concentrations to give the same detector response.

Recently, Fortier and Fritz (49) proposed a new spectrophotometric detection system for water separated by liquid chromatography. This is based on the effect of water on the equilibrium between cinnamaldehyde and cinnamaldehyde dimethylacetal in the methanol-acetonitrile eluent. Their system employed a cation-exchange column in the Li^+ form for separation, followed by a catalytic column containing cation-exchange resin in the H^+ form. Preliminary work showed that it is possible to determine water in a variety of liquid samples in about 6 to 12 minutes, depending on the length and diameter of the chromatographic column used. Excellent linear calibration plots were obtained from 0.0013% up to 3.4% water.

In the present study the method of Fortier and Fritz (49) has been improved so that only a single chromatographic column is needed. Various parameters affecting the separation are systematically studied and the experimental conditions are optimized. As a result, a much faster and more sensitive determination of water is possible. A theoretical model of the detection system is proposed and is verified by experiments. The mechanism of the detection system is now explained in detail, and the factors affecting the initial "injection" peak are elucidated. Results obtained by this single-column method show good agreement with those obtained with Karl Fischer titration method. The scope of the method is also demonstrated using a wide variety of samples.

EXPERIMENTAL SECTION

Apparatus

The chromatographic system consisted of a LKB 2150 HPLC Pump with variable flow rate from 0.01 to 5.0 ml/min, a model 7010 Rheodyne injector equipped with sample loops sized from 5 μl to 100 μl depending on the sample water content, a Spectroflow 783 Kratos UV-Vis Detector with variable detection wavelength, and a Curken strip-chart recorder. Columns of different dimensions packed with various cation-exchange resins were employed. The columns were packed on a Shandon single-piston packing pump, using upward slurry packing method. Due to the large degree of shrinking and swelling that occurs in polystyrene-divinylbenzene resins when a change in solvent occurs, it was necessary to pack the column in the same solvent used in the mobile phase. A Hamilton PRP-X300 ion-exclusion column (4.6 mm X 15 cm) and a Supelco LC-Diol column (4.6 mm X 25 cm) was also tested.

Reagents

Trans-cinnamaldehyde (99%), trimethyl orthoformate (98%), and anhydrous acetonitrile were purchased from Aldrich Chemicals (Rochester, NY) and were used without further purification. Karl Fischer grade

(anhydrous) methanol, one-component reagent for Karl Fischer titration (HYDRANAL-Composite 2, 1 ml = 2 mg H_2O), and water standards (1.00 ± 0.02 mg H_2O and 5.00 ± 0.02 mg H_2O per ml) were obtained from Fisher Scientific (Pittsburgh, PA). Aminex Q-150S, Aminex 50W-X4, and Aminex A-7 cation-exchange resins in Na^+ form were from Bio-Rad (Richmond, California) and were converted to H^+ form by equilibrating with methanol solution containing 1.0 M sulfuric acid. Polystyrene-divinylbenzene resin used to prepare the sulfonated resins with different capacities was provided by Serasep (Santa Clara, CA). All other chemicals were reagent grade or better and were used without purification. Distilled water was further purified with the Barnstead Nanopure II System before use.

Eluent and Standard Samples

Eluent was prepared simply by dissolving carefully weighed amount of cinnamaldehyde to mixture of anhydrous methanol and acetonitrile. Standard samples were prepared by adding accurately measured volumes of water to known volumes of anhydrous acetonitrile or methanol contained in vials equipped with hole caps and teflon-faced Neoprene septa (Supelco Inc, Bellefonte, PA). For maximum sensitivity and reproducibility, the eluent and all standard samples were prepared under

the protection of dried nitrogen. Once prepared, the eluent was protected from atmospheric moisture using a septum capped reservoir and a balloon filled with dry nitrogen which was connected to the reservoir through a needle. Water-saturated organic samples were prepared by shaking with excess water for 24 hours and equilibrating in a thermostat at 23 °C for another 24 hours.

Chromatographic Conditions

Unless pointed out specifically, the following experimental conditions were used throughout this study: a 2.5 cm x 2.1 mm column packed with Aminex Q-150S resin in H^+ form; an eluent of 40% methanol and 60% acetonitrile containing 1.0 mM cinnamaldehyde; a flow rate of 0.5 ml/min; a 5-μl injection loop, and a detection wavelength at 300 nm.

Functionalization of Polymeric Resins

About 5 g polystyrene-divinylbenzene resin was washed and wet with 50 ml glacial acetic acid. Excess acetic acid was filtered through a glass filter with coarse frit and the wet resin was transferred into a 100 ml round-bottom bottle. Thirty milliliters of concentrated sulfuric acid was then added to the bottle and stirred with a magnetic stir bar. After certain time period, the reaction was quenched by adding deionized

water. The resin was washed with deionized water, methanol and air-dried. The capacity of the resin is determined by adding an excess of standard NaOH solution and back titrating with standardized HCl solution. To obtain resins with very high capacity, it was necessary to heat the reaction with a oil bath thermostated at 65 °C.

Karl Fischer Titration

Karl Fischer titration was performed with a home made closed system consisted of a 10-ml semi-automatic buret, a 150-ml erlenmeyer flask and a small magnetic stir bar. The system was protected from moisture using drying tubes filled with drierite. The one-component reagent obtained from Fisher Scientific was standardized using either water standards or deionized water. A visual end-point was employed (4).

RESULTS AND DISCUSSION

The Detection System

The cinnamaldehyde added to the anhydrous methanol used to prepare the eluent has the potential of reacting with the methanol to form cinnamaldehyde dimethylacetal plus water (Equation 8):

$$C_6H_5CH{=}CH{-}CHO + 2\ CH_3OH \overset{H^+}{\rightleftharpoons} C_6H_5CH{=}CH{-}CH(OCH_3)_2 + H_2O \qquad (8)$$

However, this reaction does not occur to any extent until an acid catalyst is present. This may be the H^+-form cation exchange resin in the column, or a soluble acid added to the eluent. In the presence of a trace amount of acid, the reaction begins to occur at a noticeable rate (Figure 1). The reaction becomes effectively instantaneous at an acid concentration greater than 0.01 M. After the reaction reaches equilibrium, most of the cinnamaldehyde is converted into the acetal form, as is evidenced by the UV-Vis spectra in Figure 2. It can be seen that the spectra of cinnamaldehyde and its dimethylacetal differ significantly from each other. While cinnamaldehyde absorbs at a maxima of 285 nm ($\epsilon = 2.4 \times 10^4$), its dimethylacetal absorbs at a maxima of 250 nm ($\epsilon = 2.1 \times 10^4$). At 300 nm for instance, where cinnamaldehyde absorbs strongly, the acetal only slightly absorbs.

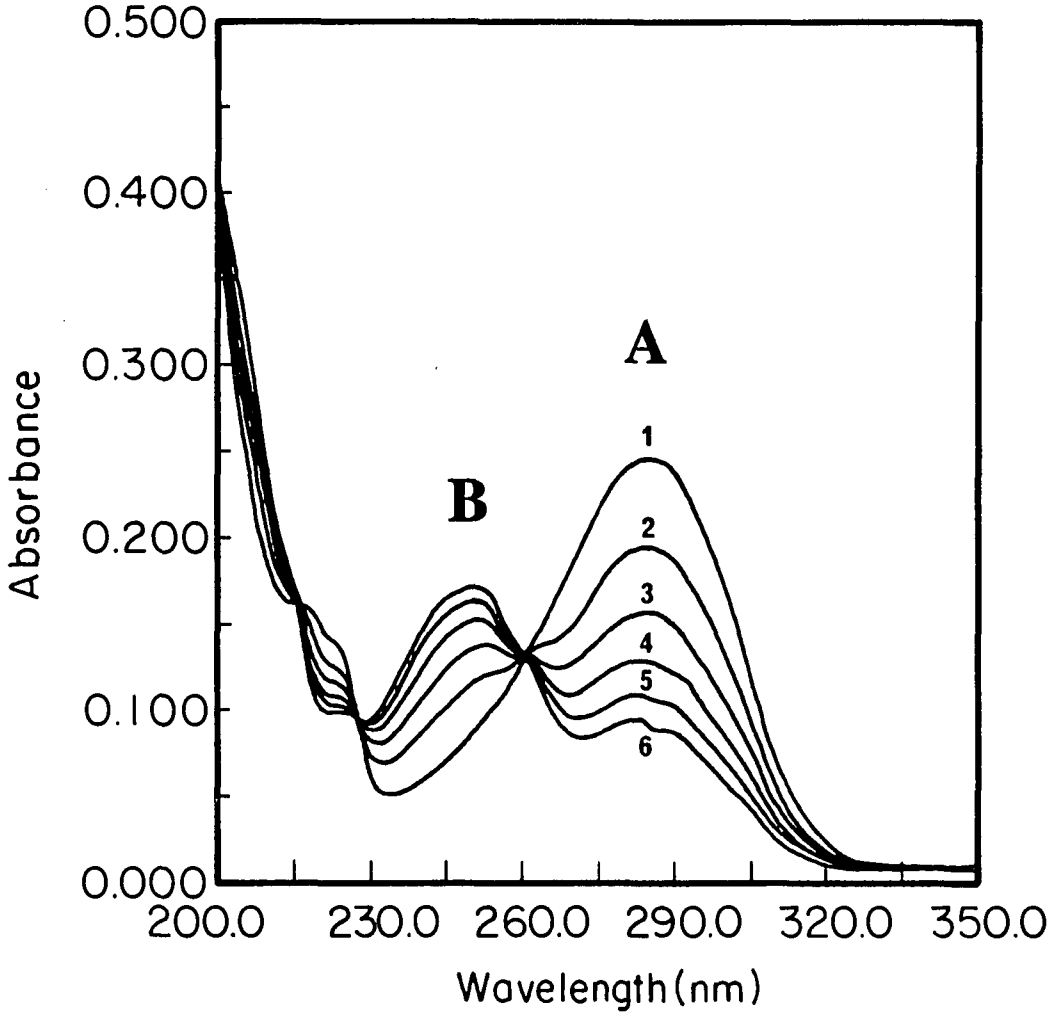


Figure 1. Time-resolved UV-Vis spectra of a methanol solution containing 0.01 mM cinnamaldehyde recorded right after adding 0.5 mM hydrochloric acid. Peak A, absorption by cinnamaldehyde; peak B, absorption by cinnamaldehyde dimethylacetal. Spectra 1 to 6 represent the scanning order. Scanning rate, 100 nm/min; cycle time, 1.5 minutes

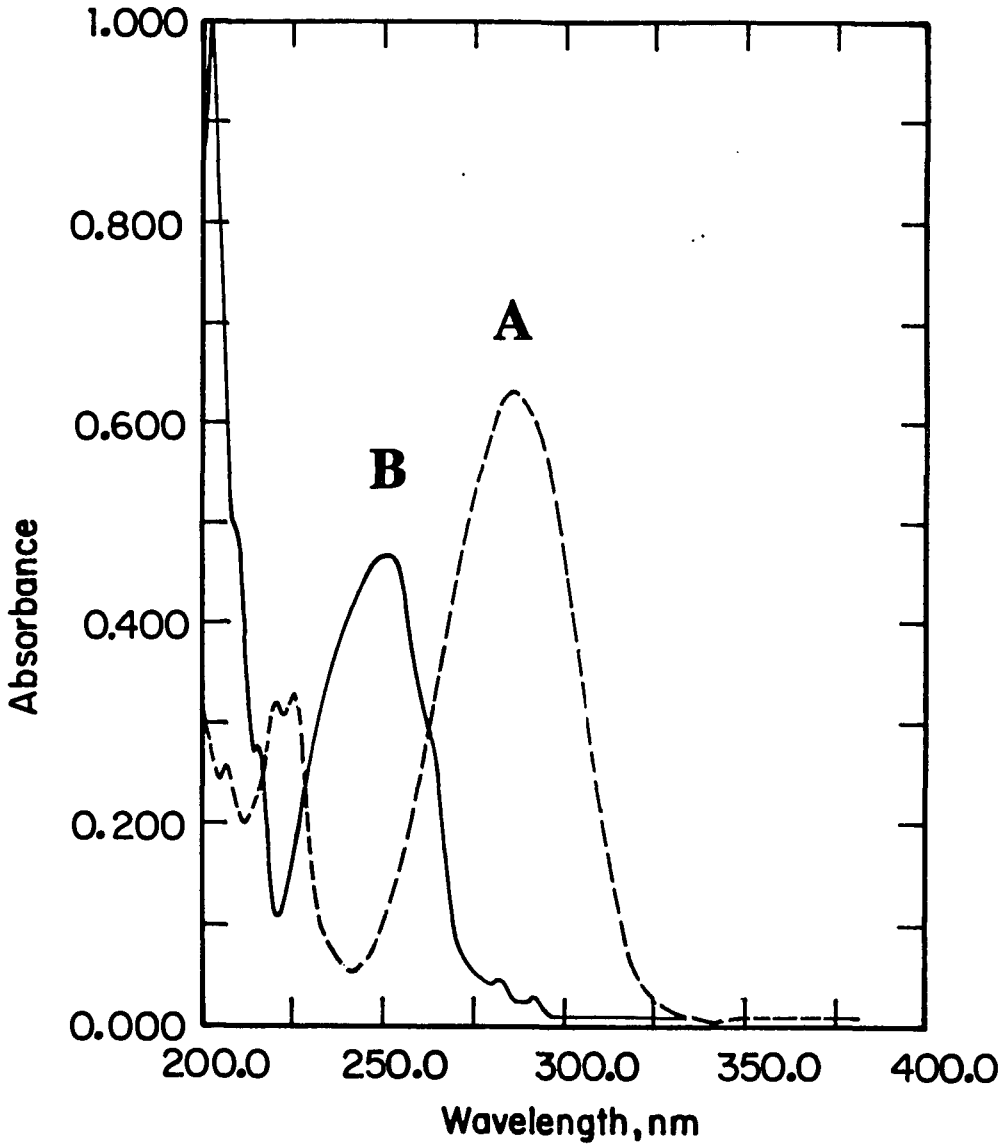


Figure 2. UV-Vis spectra obtained before and after bubbling a methanol solution containing 0.026 mM cinnamaldehyde with HCl gas for 10 seconds. A, before bubbling the HCl gas when all cinnamaldehyde remains unreacted; B, after bubbling the HCl gas when most of the cinnamaldehyde is converted to the acetal form

In a chromatographic system, a short separation column in the H^+ form is found sufficient to catalyze an instantaneous reaction. After passing through the column, the absorbance of the eluent becomes very low at a wavelength (e.g., 300 nm) where only the cinnamaldehyde absorbs strongly. This is because that the majority of the cinnamaldehyde is converted to the acetal. However, when a significant amount of water is introduced into the chromatographic system along with the sample, the equilibrium (Equation 8) will be shifted back, forming more cinnamaldehyde. This results in an increase in the detector signal which is proportional to the concentration of water in the sample. This change in detector signal serves as the indirect detection of water in this method.

Equilibrium constant

The equilibrium constant for the following reaction was measured by adding varying concentrations of water to the eluent (in the presence of 0.01 M H^+) and measuring the concentrations of cinnamaldehyde and its dimethylacetal spectrophotometrically:

$$\underset{(b)}{H_2O} + \text{cinnamaldehyde acetal} \xrightleftharpoons{H^+} 2\ \underset{(a)}{CH_3OH} + \text{cinnamaldehyde} \qquad (9)$$

The measurements were made at 280 nm, where both cinnamaldehyde and its dimethylacetal absorb appreciably. First, the absorbance of the eluent ($A_a°$) was measured before addition of an acid catalyst when all of the cinnamaldehyde remains unreacted. Then the absorbance ($A_b°$) is measured after acid catalysis when all of the cinnamaldehyde has been converted to the acetal form. From Beer's law:

$$A_a° = \epsilon_a \, l \, C° \tag{10}$$

$$A_b° = \epsilon_b \, l \, C° \tag{11}$$

Here ϵ is the extinction coefficient, l is the path length of the detector cell, and $C°$ is the cinnamaldehyde concentration added to the eluent.

Next, varying amounts of water were added to the eluent in the presence of an acid catalyst and the total absorbance (A_{tot}) was measured. From Beer's law:

$$A_{tot} = A_a + A_b = \epsilon_a l[a] + \epsilon_b l[b] \tag{12}$$

$$= \epsilon_a l[a] + \epsilon_b l\{C° - [a]\} \tag{13}$$

$$= (\epsilon_a l - \epsilon_b l)[a] + A_b° \tag{14}$$

Here, [a] and [b] are the equilibrium concentrations of cinnamaldehyde and its dimethylacetal in the eluent, respectively. Combining these equations, we have

$$[a] = \frac{A_{tot} - A^{\circ}_{b}}{\epsilon_a l - \epsilon_b l}, \quad \text{and} \quad [b] = C^{\circ} - [a] = \frac{A^{\circ}_{a} - A_{tot}}{\epsilon_a l - \epsilon_b l} \tag{15}$$

The equilibrium constant (K) for Equation 2 is:

$$K = \frac{[a]}{[b][H_2O]} = \frac{A_{tot} - A^{\circ}_{b}}{(A^{\circ}_{a} - A_{tot})[H_2O]} \tag{16}$$

A value of (5.3 ± 0.4) X 10^{-4} mM^{-1} was determined for the equilibrium constant, K.

As indicated by the equilibrium constant, only a small fraction of the water from the sample is consumed in shifting the cinnamaldehyde dimethyl acetal-cinnamaldehyde equilibrium (Equation 9) to the right. Most of the water remains unreacted and emerges from the column as a distinct peak with a longer retention time than the bulk of the sample. It is believed that water is retarded mainly via an ion-exclusion mechanism (50,51), with a possible contribution from hydrogen bonding interaction (52).

Theoretical considerations

Rearrangement of Equation 16 gives

$$A_{tot} = \frac{A_a^\circ \, K[H_2O] + A_b^\circ}{K[H_2O] + 1} \qquad (17)$$

So long as the water concentration $[H_2O]$ is not too high, the denominator is approximately equal to one and Equation 17 is essentially linear. However, the detector response (A_{det}) depends on the difference in absorbance of the eluent and sample, so Equation 17 can be written:

$$A_{det} = \Delta A_{tot} = A_a^\circ \, K \left\{ [H_2O]_{sample} - [H_2O]_{eluent} \right\} \qquad (18)$$

Introducing E as a factor of column and elution efficiency and using an eluent with low but constant water concentration, the following equation is obtained for detector response:

$$A_{det} = A_a^\circ \, K \, E \, [H_2O]_{sample} - \text{Constant}$$

$$= \epsilon_a l \, C^\circ \, K \, E \, [H_2O]_{sample} - \text{Constant} \qquad (19)$$

From Equation 19, the detector response is linearly proportional to the extinction coefficient of cinnamaldehyde (ϵ_a), the path length of the detector cell (l), the initial cinnamaldehyde concentration added to the eluent (C°), the apparent equilibrium constant (K), the column and

elution efficiency factor (E), and most importantly, the water concentration in the sample ($[H_2O]_{sample}$). This equation predicts a linear calibration curve which is desirable for an analytical procedure. As discussed later, all the experiments performed support such a relationship.

Detection Wavelength

An earlier paper (49) recommended 310 nm as the wavelength for the detection of water. However, according to Equation 19, the sensitivity is proportional to the extinction coefficient of cinnamaldehyde (ϵ_a). This is confirmed experimentally by measuring the peak height of the same sample at wavelengths between 270 and 310 nm (Figure 3). The sensitivity was found to be much better at 300 nm than at 310 nm. A detection wavelength of 290 nm gave an even higher sensitivity, but the background absorbance was also much higher. Therefore, 300 nm was chosen as the detection wavelength for subsequent studies.

Eluent

Effect of cinnamaldehyde concentration

Equation 19 predicts that increasing concentrations of cinnamaldehyde in the eluent should increase the detector signal for

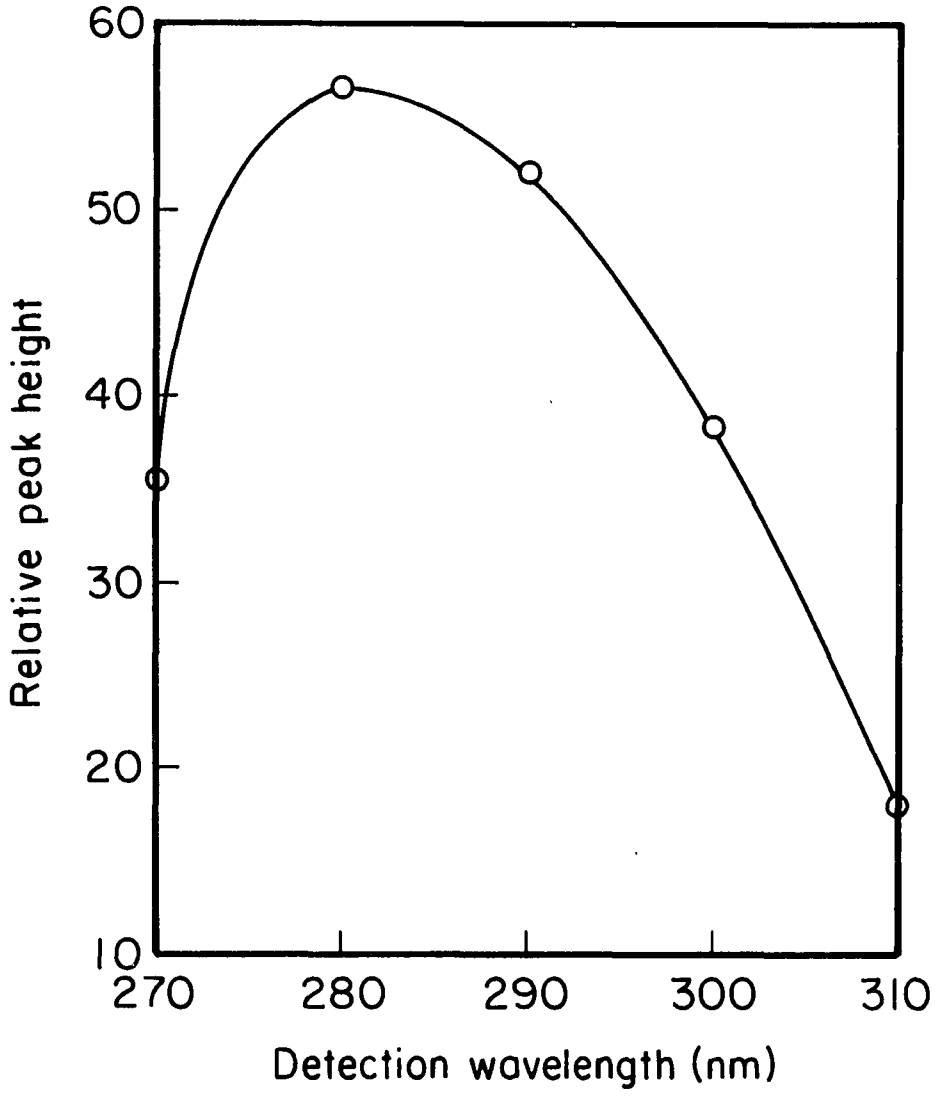


Figure 3. Dependance of the water peak height on the detection wavelength. Sample, 1.0% H_2O in anhydrous acetonitrile. Other conditions are given in the text

samples containing a fixed concentration of water. This is indeed the case, as is shown by the chromatograms in Figure 4. A plot of peak height against cinnamaldehyde concentration in the eluent is linear and passes through the origin for eluent concentration points of 0.5, 1.0, 2.0, and 5.0 mM in cinnamaldehyde (Figure 5).

Effect of eluent composition

Addition of another organic solvent to the methanol eluent increases the retention time for the water peak and also broadens the peak somewhat. Figure 6 shows that methanol-acetonitrile eluents give longer retention times for water than methanol alone. The detector response (peak height) also increases with increasing proportions of acetonitrile in the eluent. This can be explained by shifting the detection equilibrium farther to the right as the concentration of methanol in the eluent is decreased (Equation 20).

$$H_2O + \text{cinnamaldehyde acetal} \underset{\longleftarrow}{\overset{H^+}{\longrightarrow}} 2\ CH_3OH + \text{cinnamaldehyde} \qquad (20)$$

In another word, the dilution of the methanol eluent with an organic solvent increases the apparent equilibrium constant K:

$$K = \frac{[a]}{[b]\ [H_2O]} \qquad (21)$$

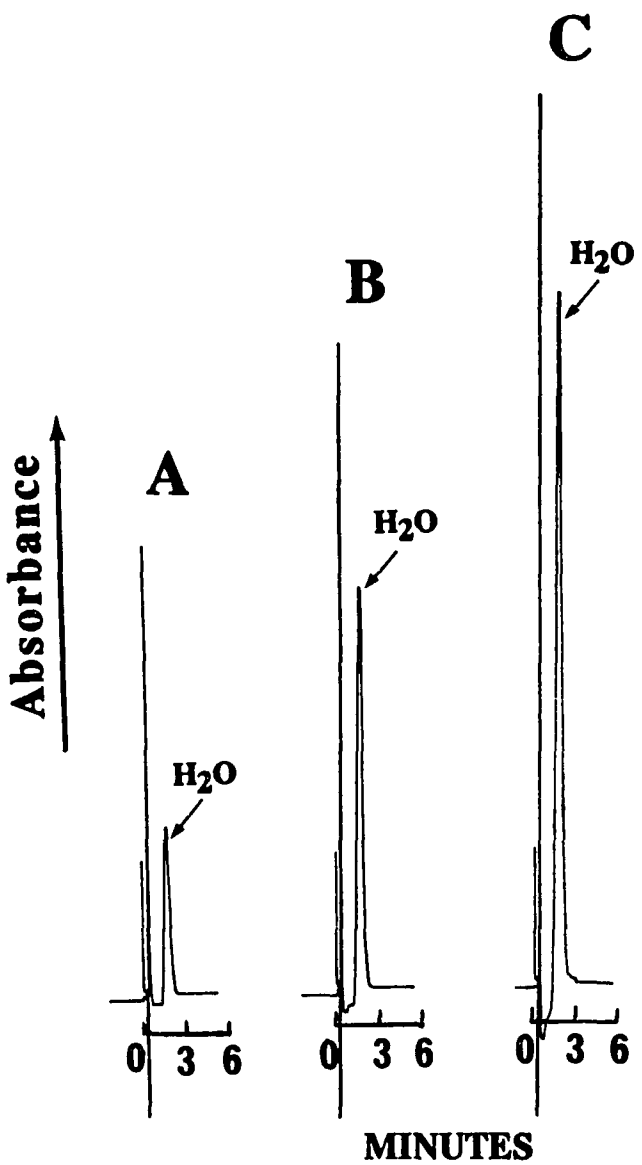


Figure 4. Chromatograms of the same sample obtained using eluents with different initial cinnamaldehyde concentration added. Initial cinnamaldehyde concentration: A, 0.5 mM; B, 1.0 mM; C, 2.0 mM. Sample, 1.0% H_2O in anhydrous acetonitrile. Other conditions are given in the text

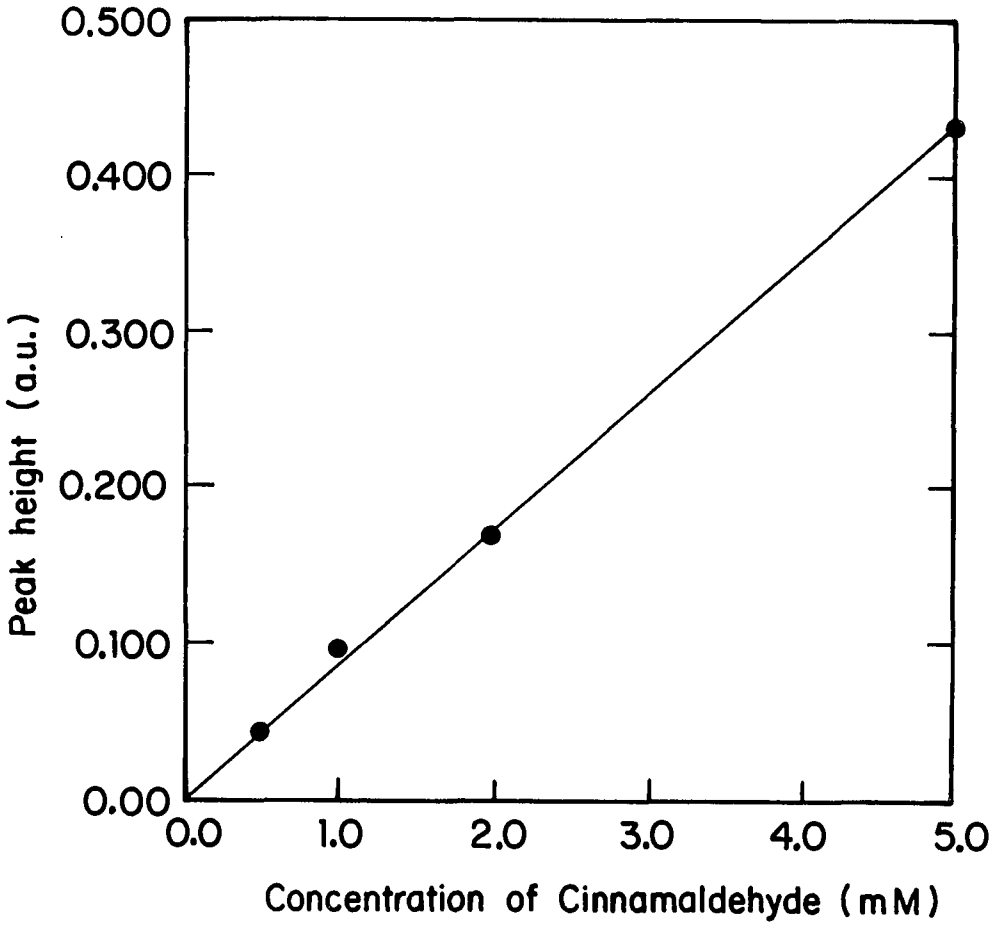


Figure 5. Plot of water peak height against the initial cinnamaldehyde concentration added to the methanol eluent. Other conditions are the same as given in Figure 4

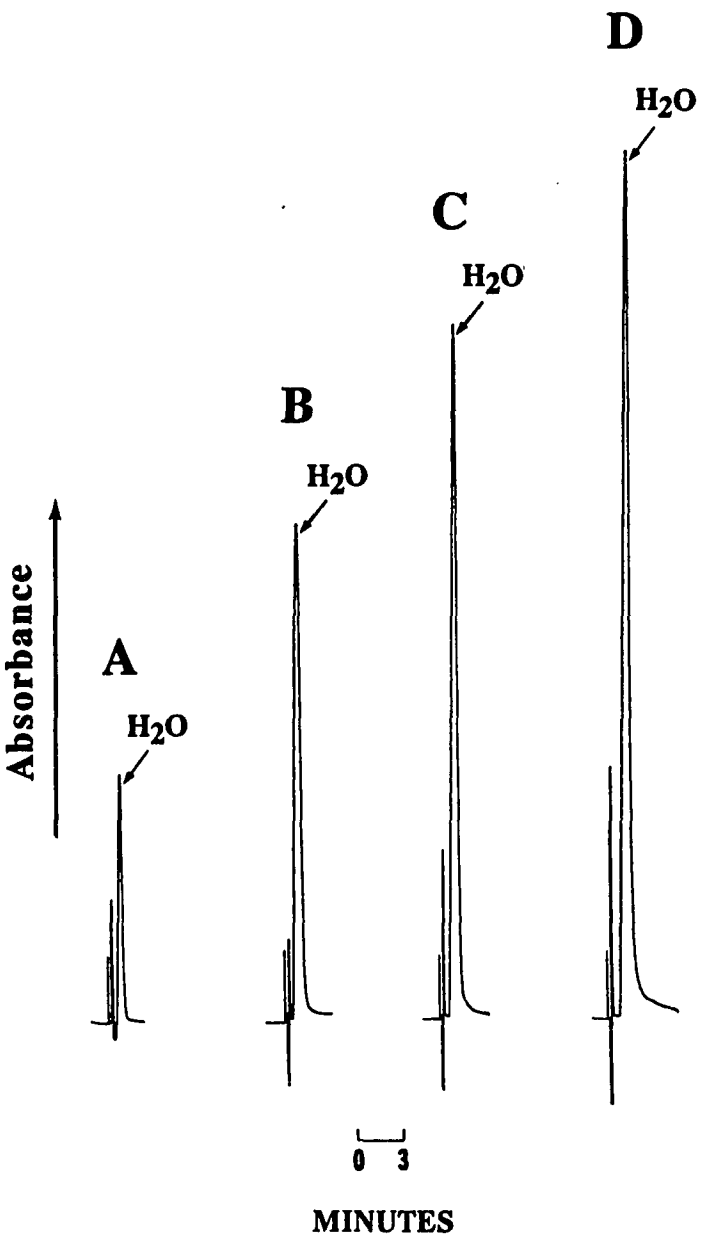


Figure 6. Chromatograms of the same sample obtained with eluents containing different proportions of acetonitrile. A, 100% methanol; B, 60% methanol and 40% acetonitrile; C, 40% methanol and 60% acetonitrile; D, 20% methanol and 80% acetonitrile. Sample, 1.0% H_2O in anhydrous acetonitrile. Other conditions are given in the text

As a result, the sensitivity of the detection system increases according to Equation 19. The best composition appears to be 40% methanol and 60% acetonitrile and was used in most experiments. At 20% methanol and 80% acetonitrile, the eluent baseline becomes unsteady.

Mixtures of three different solvents with methanol were compared as eluents, as shown in Figure 7. Methylene chloride increases the retention time more than acetonitrile or tetrahydrofuran and the water peak is somewhat broader. Among the three solvents, acetonitrile provides the best result in terms of peak shape and sensitivity. For this reason, acetonitrile was used as the second solvent in the eluent.

Flow rate

As would be expected, a faster flow rate lowers the retention time but also reduces the sensitivity owing to a shorter reaction time (Figure 8). It seems that compromise has to be made between the separation speed and the detection sensitivity. A flow rate of 0.5 ml/min is recommended for most separations performed on a 2.5 cm x 2.1 mm column.

Trimethyl orthoformate as the drying reagent

One common problem encountered by most of the water-determining

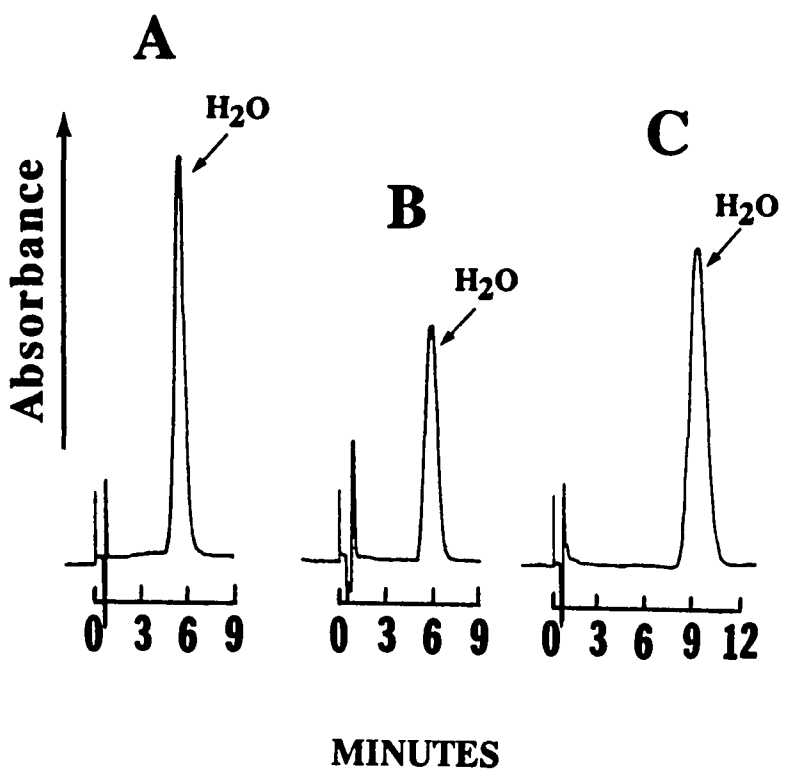


Figure 7. Chromatograms of the same sample obtained with eluents containing portions of different organic solvents. A, 60% methanol and 40 % acetonitrile; B, 60% methanol and 40 % tetrahydrofuran; C, 60% methanol and 40 % methylene chloride. Column: a 15 cm x 4.6 mm Li^+-form separation column and a 2.5 cm x 2.1 mm H^+-form catalyst column were used (see two-column method in Section II). Sample, 1.0 % H_2O in anhydrous acetonitrile

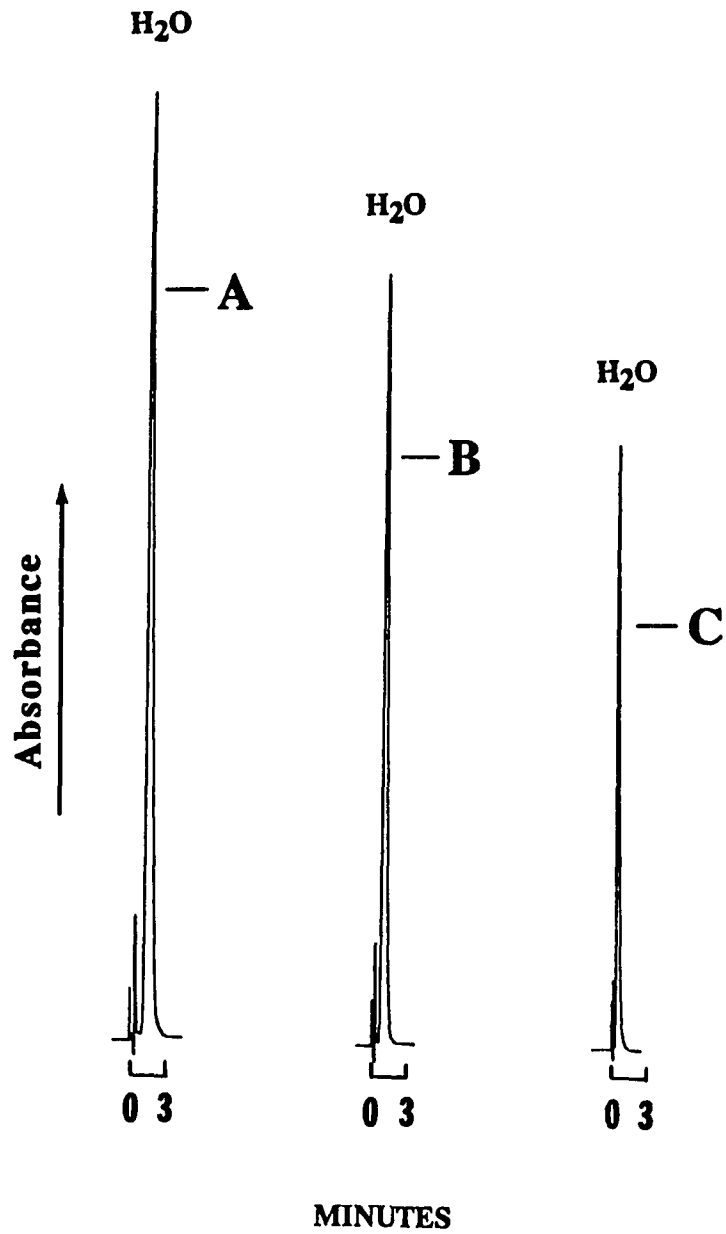


Figure 8. Chromatograms of the same sample obtained using different flow rates. A, 0.5 ml/min; B, 0.8 ml/min; C, 1.0 ml/min. Sample, 1.0% H_2O in anhydrous acetonitrile; Column, 2.5 cm x 2.1 mm packed with Aminex A-7 resin. Other conditions are given in the text

methods is the background water in the solvents or eluents. A popular way to remove water is to use a drying reagent such as activated molecular sieves, sodium ethoxylate, or calcium hydride. The drying procedure usually requires distillation of the solvents in a closed system after equilibration with the drying reagents for more than 24 hours. Solvents dried with these reagents normally have a water content ranged from several tens to several hundreds ppm and are satisfactory for most applications. However, these water levels are still too high if the determination of low ppm water is required.

This problem is now solved by using trimethyl orthoformate (TMOF) as the drying reagent. TMOF and water undergo the following reaction:

$$HC(OMe)_3 + H_2O \xrightarrow{H^+} HCOOMe + 2\ MeOH \qquad (22)$$

This reaction is virtually instantaneous in the presence of low concentration of an acid (e.g., H_2SO_4) and is very complete as will be discussed in Section III. The two products formed, methylformate and methanol, present no side effects to the eluent and therefore no separation is required after the reaction.

The drying procedure calls for a dropwise addition of TMOF to the eluent while monitoring the drop of the baseline at 300 nm using a UV-Vis detector. A leveled baseline indicates the complete consumption of

water in the eluent. Figure 9 shows a large drop of the baseline after the addition of appropriate amount of TMOF to an eluent containing 0.01 M H_2SO_4. More than two fold increase in sensitivity was also obtained after drying of the eluent with TMOF (Figure 10).

Using the above drying procedure, water concentration as low as 26 ppm can be easily determined (Figure 11).

Column

Dimensions

Aminex Q-150S columns (H^+-form) of varying dimensions were tried. Columns with a 2.1 mm inside diameter worked the best. Columns of wider diameter gave lower detection sensitivity (Figure 12).

As discussed previously, increasing the flow rate speeds up the separation but also reduces the sensitivity. It seems that some compromise has to be made between the separation speed and the sensitivity. This problem can be solved by using a short column and a lower flow rate. Figure 13 Shows that a 2.5 cm x 2.1 mm column coupled with a 0.5 ml/min flow rate gives a much higher sensitivity while maintaining fast separation speed. This increase in sensitivity is probably due to two factors: (1) a better column efficiency resulted from a short, nicely packed column; and (2) less peak broadening as a

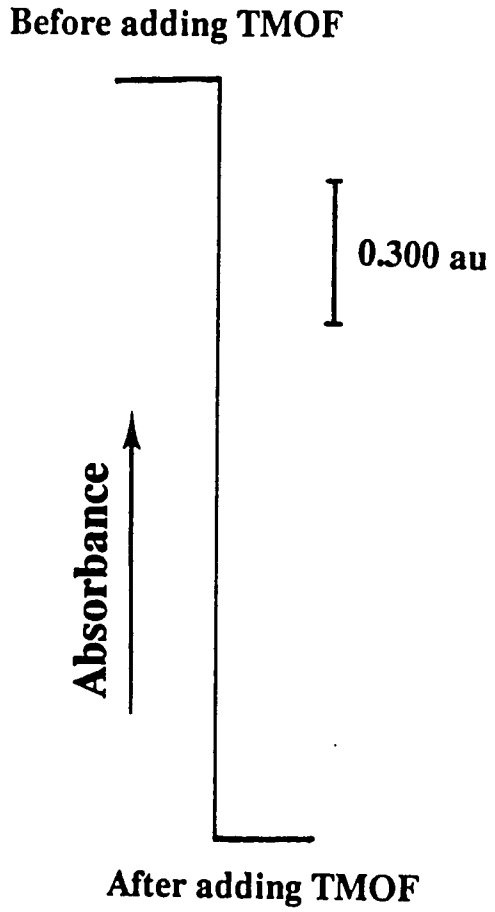


Figure 9. Change in eluent baseline before and after drying the eluent with TMOF. Sample, 1.0% H_2O in anhydrous acetonitrile. Other conditions are given in the text

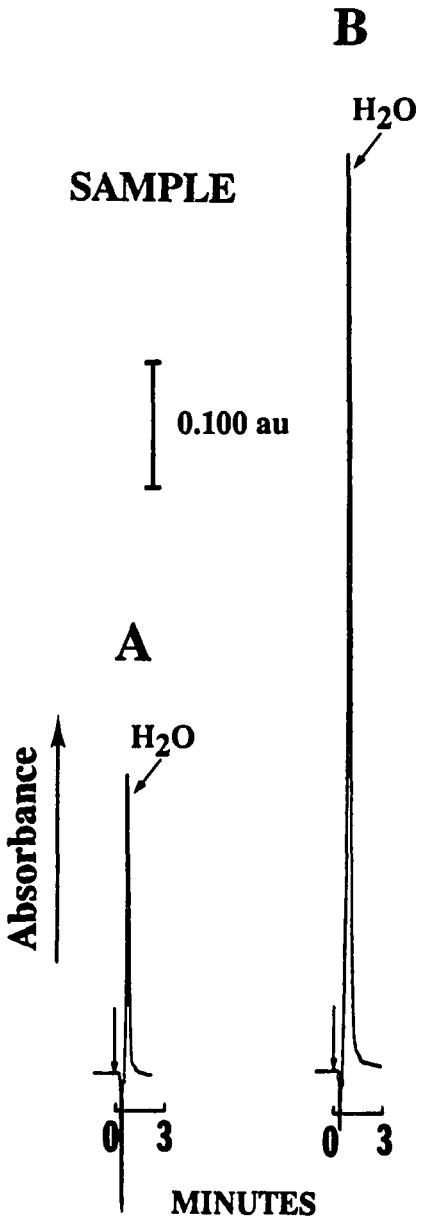


Figure 10. Chromatograms of the same sample obtained before and after drying the eluent with TMOF. Other conditions are the same as given in Figure 9

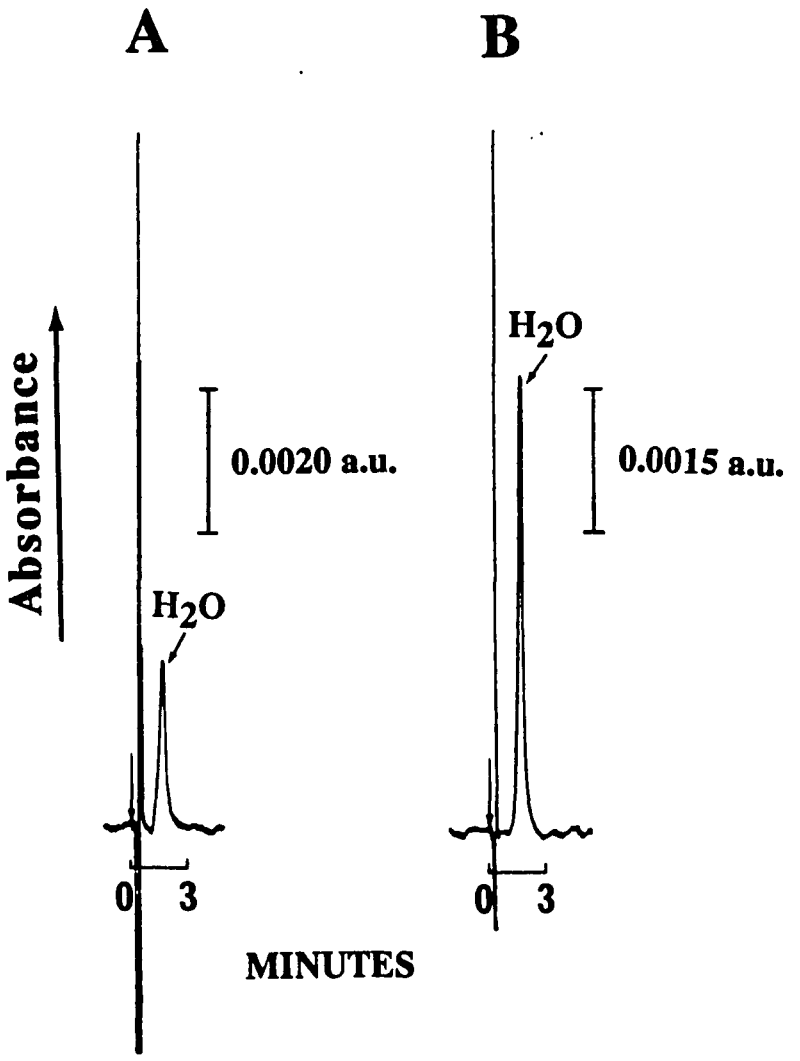


Figure 11. Determination of water in anhydrous organic compounds using eluent dried with TEOF. A, 26 ppm H_2O in anhydrous decehydronaphthalene; B, 46 ppm H_2O in anhydrous acetonitrile. Other conditions are given in the text

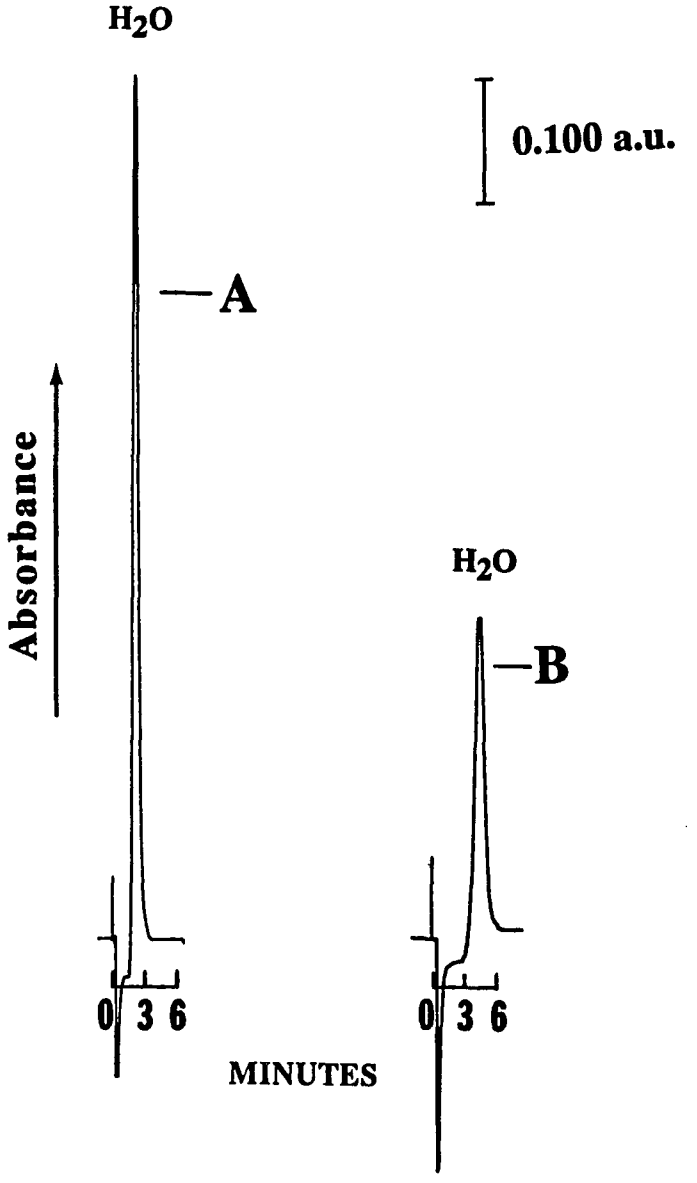


Figure 12. Chromatograms of the same sample obtained using two columns of different inside diameters. A, 15 cm x 2.1 mm i.d.; B, 15 cm x 4.6 mm i.d. Sample, 1.0% H_2O in anhydrous acetonitrile. Other conditions are given in the text

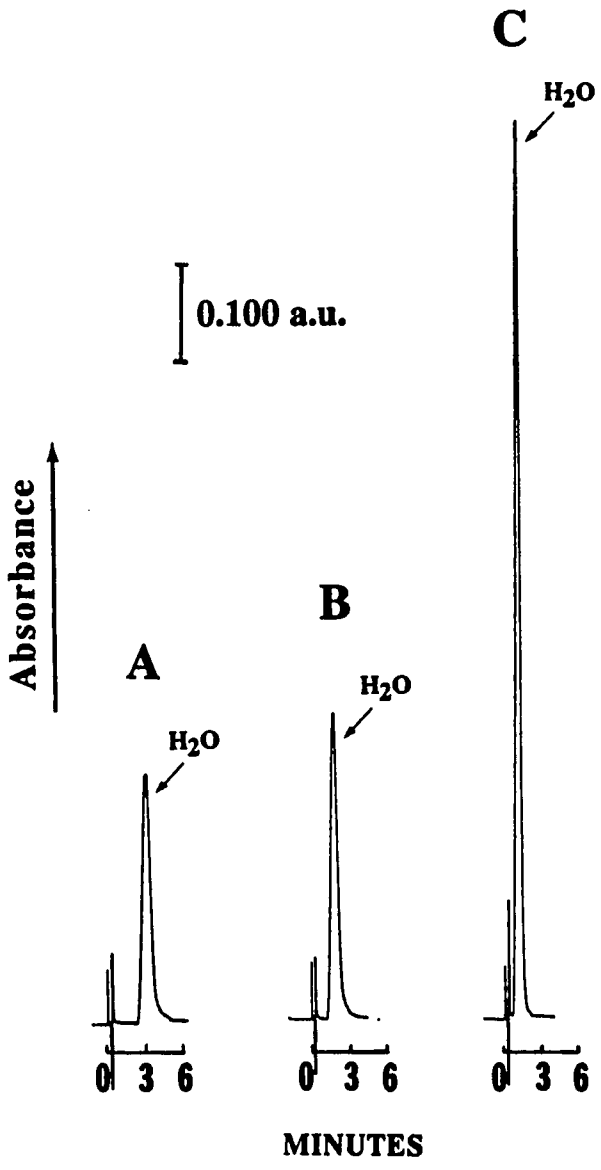


Figure 13. Chromatograms of the same sample obtained using columns of different lengths. A, 15 cm x 2.1 mm column and 1.0 ml/min flow rate; B, 10 cm x 2.1 mm column and 1.0 ml/min flow rate; C, 2.5 cm x 2.1 mm column and 0.5 ml/min flow rate. Sample, 1.0% H_2O in anhydrous acetonitrile. Other conditions are given in the text

result of a narrow and shorter column.

Properties of the resin

Several cation-exchange resins were tried as the packing for the separation column. Among them, Aminex Q-150S, Aminex 50W-X4, and Aminex A-7 resins worked very well. A Serasep polymeric resin (polystyrene-divinylbenzene) functionalized with sulfonic acid groups was found to give the highest sensitivity (Figure 14). It is a gel-type resin, which appears to be a desirable property for chromatographic separation of water from other substances (48). Some of the physical characteristics of these resins are listed in Table III. Among these properties, the size of the resin particles seems to play the most significant role in determining the water peak shape and detection sensitivity.

Resins of varying capacity were also prepared by functionalization of the Sarasep resin. Figure 15 shows that the retention time increases with increasing capacity. This is presumably caused by an increased degree of hydrogen bonding interaction. While moderate capacities give similar sensitivities, high capacities result in significant band broadening and reduces the sensitivity.

Hamilton PRP-X300 is said to be a good column for ion-exclusion chromatography, but it gave no separation at all for water under the

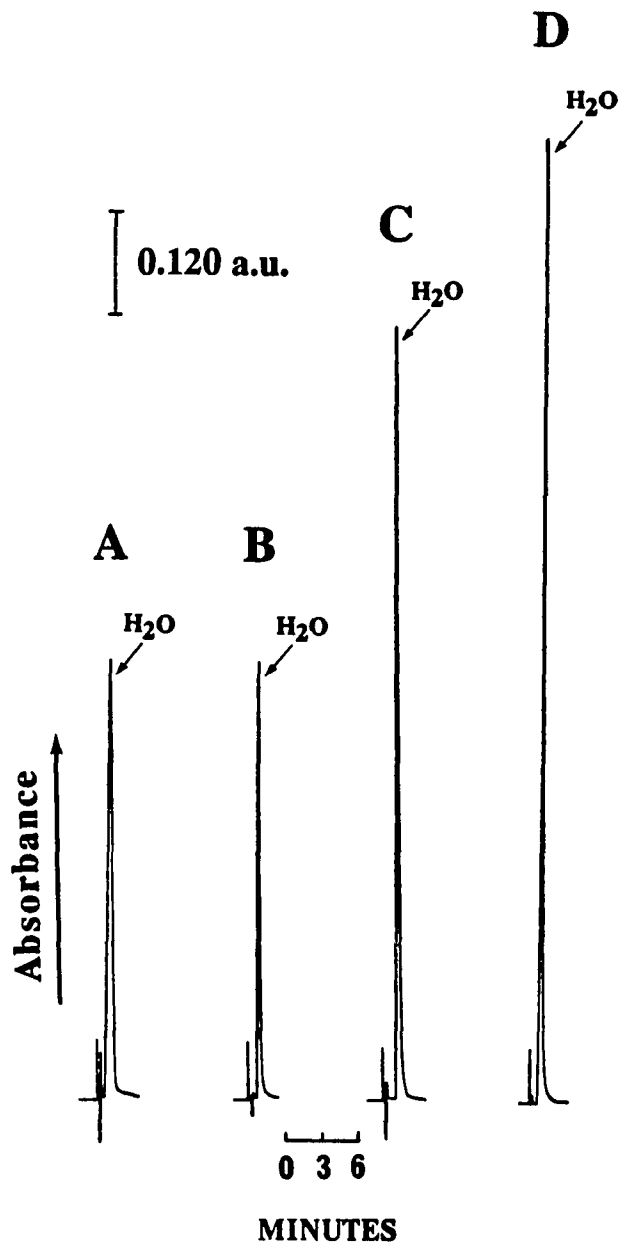


Figure 14. Chromatograms of the same sample obtained using columns packed with different cation-exchange resins. A, Aminex Q-150S; B, Aminex 50W-X4, C, Aminex A-7; D, Functionalized Serasep polystyrene-divinylbenzene resin. Sample, 1.0% H_2O in anhydrous acetonitrile. Other conditions are given in the text

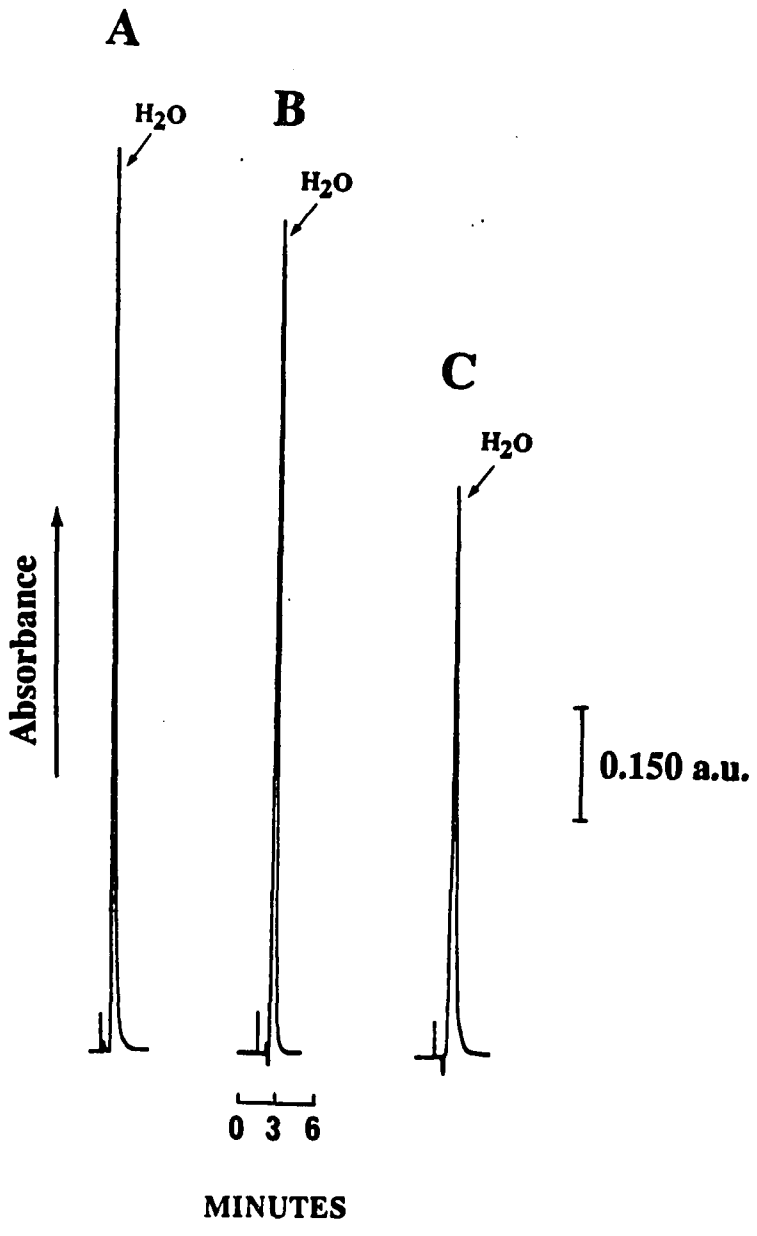


Figure 15. Chromatograms of the same sample obtained using columns packed with resins of different capacities. Capacity of the resin: A, 0.64 meq/g; B, 1.51 meq/g; C, 2.79 meq/ml. Sample, 1.0% H_2O in anhydrous acetonitrile. Other conditions are given in the text

Table III. Some physical characteristics of the cation-exchange resins used in the the experiments

Resin type	Degree of cross-linking	Particle size	Capacity
Aminex Q-150S	8%	28 ± 7 μm	1.7 meq/g
Aminex 50W-X4	4%	25 ± 5 μm	1.2 meq/g
Aminex A-7	8%	7 - 11 μm	1.7 meq/g
Serasep[a]	8%	~ 10 μm	0.64 meq/g

[a]Functionalized from the Serasep polystyrene-divinylbenzene resin

conditions we used. Perhaps this is because the Hamilton resin is macroporous and not a gel. A silica-based diol column also gave no separation of water. A slight displacement of the water peak (longer retention) was due to a short Aminex Q-150S (H^+-form) post column used as the catalyst.

Calibration Curves

Standards were prepared by adding carefully measured amounts of water to portions of dry acetonitrile. After chromatographic separation, linear plots of peak height against water concentration were

obtained with excellent correlation coefficients (0.9999 - 0.99999) for linear regression. Once again, this experimental result shows good agreement with the prediction by Equation 19.

Figure 16 shows a typical calibration plot for water that exhibits excellent linearity (r = 0.99999) over three orders of magnitude in water concentration. Figure 17 shows a linear calibration curve for samples containing higher concentrations of water.

Injection Peaks

Typical chromatograms for the determination of small amounts of water in organic liquids are shown in Figure 18. In each case there is an injection peak that occurs at the column dead time. This is followed by the water peak which has a retention time of 1.0 to 2.0 minutes, depending on the chromatographic conditions.

The source and magnitude of injection peaks was investigated. This was done by injecting samples of four organic liquids, each containing a small amount of water, into a series of eluents containing (1) methanol only, (2) methanol plus 1 mM cinnamaldehyde, and (3) methanol containing 5 mM cinnamaldehyde. The results are summarized in Table IV.

The results obtained with methanol only show that absorbance of the sample matrix can contribute to the injection peak. In this regard it

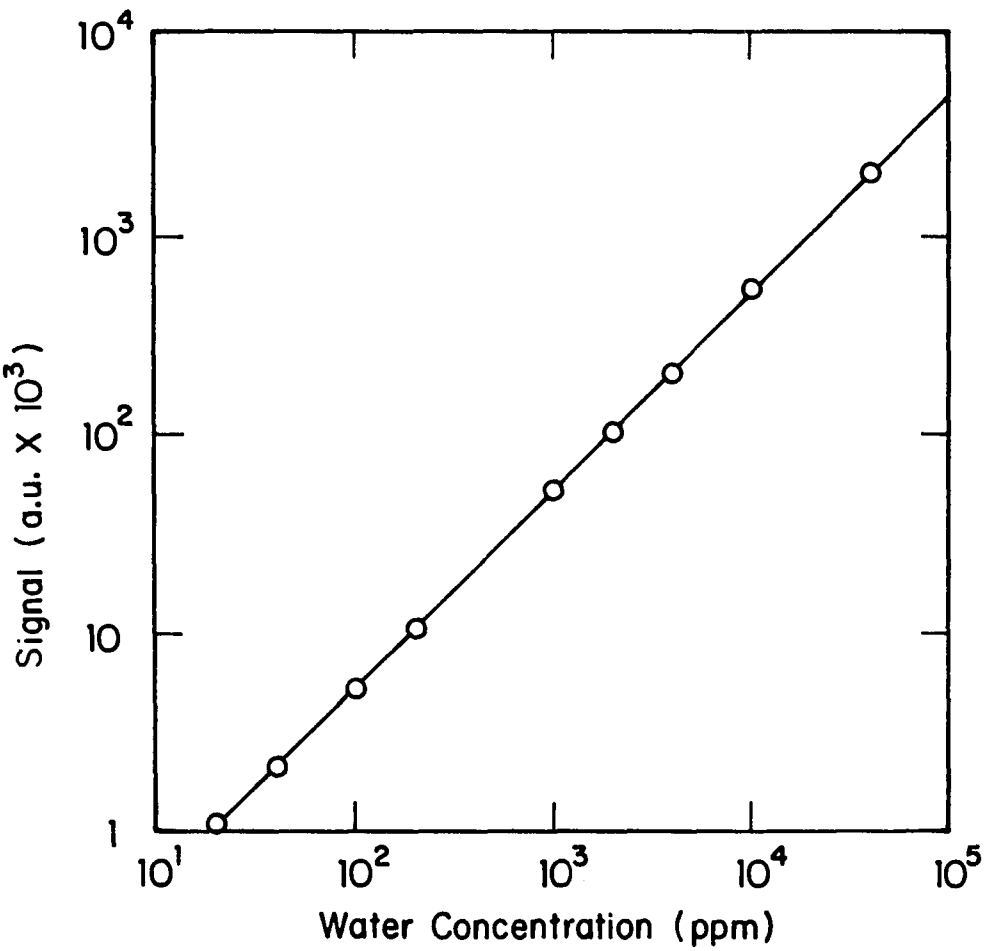


Figure 16. Calibration curve in the low to medium range of water content. A 2.5 cm x 2.1 mm column packed with Aminex Q-150S resin in H^+ form and a 50-μl injection loop was used. Other conditions are given in the text

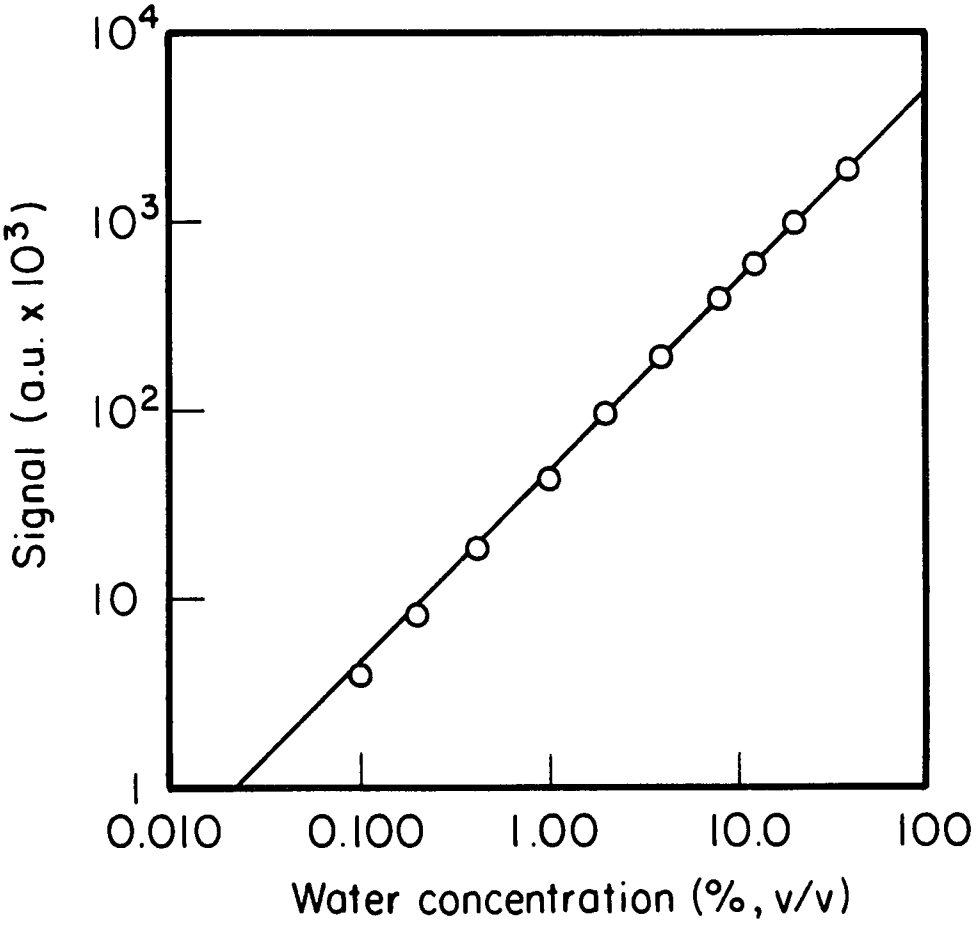


Figure 17. Calibration curve in the medium to high range of water content. A 5-μl injection loop was used. Other conditions are the same as in Figure 16

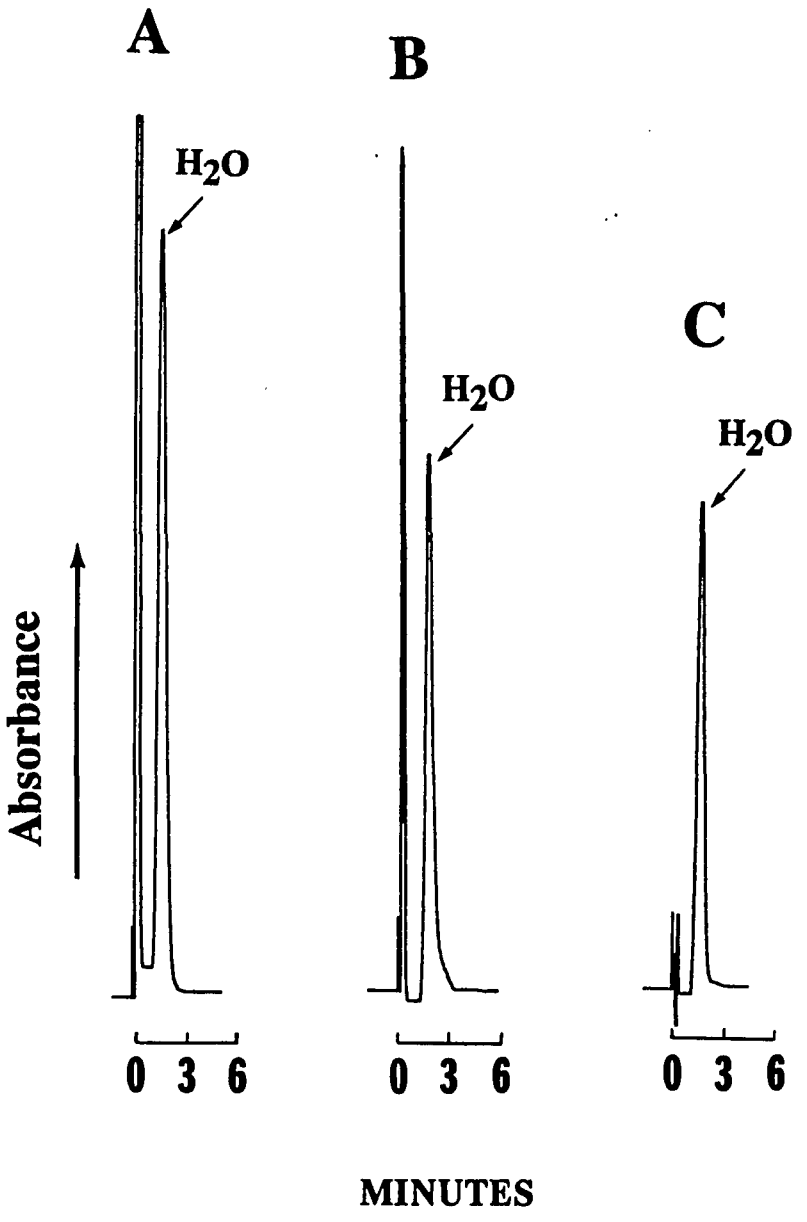


Figure 18. Determination of water in various samples. A, 0.170% H_2O in furan; B, 0.184% H_2O in 1,2-dichloroethylene; C, 1.24% H_2O in ethyl ether. Other conditions are given in the text

Table IV. Summary of injection peaks

Sample	Injection Peak		
	MeOH only	1 mM aldehyde	5 mM aldehyde
Methanol	None	Negative gap	Larger negative gap
Acetonitrile	Positive	Positive negative gap	Larger positive larger negative gap
Toluene	Large positive	Very large positive small negative gap	Very large positive larger negative gap
Hexane	Large positive	Larger positive small negative gap	Almost no positive large negative gap

should be recalled that the UV-Vis detector is very sensitive. Additional contributions to injection peaks are noted as increasing concentrations of cinnamaldehyde are added to the methanol eluent. These contributions can be explained by assuming that cinnamaldehyde can partition into the resin gel from the eluent. The injection of a sample (which contains no cinnamaldehyde) causes some of the aldehyde to come from the gel back into the liquid stream and thereby contribute to the injection peak. After the sample zone has passed, some aldehyde goes back into the resin gel from the eluent, causing a negative gap in the chromatogram.

Validation of the Method

Quantitation

The calibration curve in Figure 16 and 17 only shows peak height as a function of added water and does not account for the water already in the sample matrix and in the eluent itself.

A calibration curve of peak height vs. the total water in the standards was prepared with the aid of two standards (Fisher Scientific) certified to contain 1.00 ± 0.02 mg and 5.00 ± 0.02 mg water per ml of sample. The resulting calibration plot has the same slope as that with added water, but the intercept is different. Manipulation of these two

plots showed that the acetonitrile used to prepare the standards contained 25 ppm water. The methanol eluent was calculated to contain 18 ppm water.

Similar experiment was performed with the eluent dried by TMOF. No appreciable water in the eluent was detected.

Comparison of the LC method and Karl Fischer titration method

Samples of several organic liquids were carefully saturated with water by equilibration in a thermostat at 23°C after shaking for 24 hours. The water content of the organic phase was then determined by both the chromatographic analysis and Karl Fischer titration performed in triplicate. These results are summarized and compared with literature values in Table V. The results obtained with the LC method compare favorably to those obtained with KF titration. Some interpolation is required as the literature values are reported for slightly different temperatures than that used for the chromatographic determinations. Nevertheless, both the chromatographic and KF titration results are mostly in good agreement with the literature values.

The relative standard deviation (R.S.D.) of the LC method is no more than 5% for all the samples analyzed. Generally, the R.S.D. for the LC method and KF titration method was found similar for the same sample.

Table V. Summary of water solubility in various organic compounds

Organic compound	Solubility of Water (w/w)		
	Found (23°C)		Reported
	LC method	KF titration	
Benzene	0.0563 ± 0.0005	0.055 ± 0.001	0.053 (20°C)[53] 0.066 (30°C)[53]
Furan	0.170 ± 0.001	0.182 ± 0.001	0.141 ± 0.005 (20°C)[54]
Methylene chroride	0.154 ± 0.002	0.157 0.001	0.14 (20°C)[55] 0.167 (25°C)[56]
Chloroform	0.088 ± 0.001	0.091 ± 0.001	0.114 ± 0.004 (15°C)[57]
1,2-Dichloride ethylene	0.184 ± 0.001	0.163 ± 0.001	0.17 (20°C)[55] 0.187 (25°C)[56]
Ethyl ether	1.24 ± 0.01	1.27 ± 0.01	1.2 (20°C)[58] 1.26 ± 0.02 (RT)[57]
Carbon tetrachloride	0.022 ± 0.001	0.022 ± 0.001	0.035 ± 0.003 (15°C)[57] 0.0075 ± 0.0005 (20°C)[54]

Response Factor

A response factor (RF) is defined as follows

$$RF = \frac{\text{signal in absorbance units at 300 nm}}{0.1\%\ H_2O \text{ in sample}} \qquad (23)$$

A RF of 0.11 a.u./0.1% H_2O has been achieved with 1.0 mM cinnamaldehyde in the eluent and a 5-μl sample loop. This is 37 times greater than the RF obtained with the earlier method (49).

Detection Limit

The detection limit depends on the water content of the eluent as well as the RF. The lowest detection limit achieved experimentally with a 5-μl sample loop and an eluent dried with TEOF was 26 ppm of water (Figure 11). Although a sample containing low enough water content was not found to measure the actual limit of detection, it was estimated as 2 ppm (with a signal to noise ratio of 3) based on the baseline noise.

Optimized Chromatographic Conditions

Although columns packed with Aminex A-7 and functionalized Serasep resins were found to give better sensitivity than columns packed with Aminex Q-150S resin, the later was still used in most experiments

because of its commercial availability and low cost. The optimized chromatographic conditions are summarized as following: a 2.5 cm x 2.1 mm column packed with Aminex Q-150S resin in H^+ form; an 40% methanol-60% acetonitrile eluent containing 1.0 mM cinnamaldehyde; a 5-μl sample size; a 0.5 ml/min flow rate; and a detection wavelength at 300 nm. With such a condition, the water peak usually comes out in about 1.5 minute.

Fast Separation of Water

A separation can be completed in less than 0.5 minute if it is required. Figure 19a shows a chromatographic determination of 1.0% water in acetonitrile on a 10 cm x 2.1 mm H^+-form column using methanol eluent containing 1 mM cinnamaldehyde. Figure 19b shows the same separation on a 2.5 cm x 2.1 mm column at a flow rate of 0.5 ml/min, using an eluent containing 40% methanol and 60% acetonitrile. The retention time for the water peak is only 1.0 min and the peak height is more than 2 times higher than in Figure 19a. Figure 19c shows a separation under the same conditions as Figure 19b except that the flow rate is doubled. The water peak now has a retention time of only 0.5 minute.

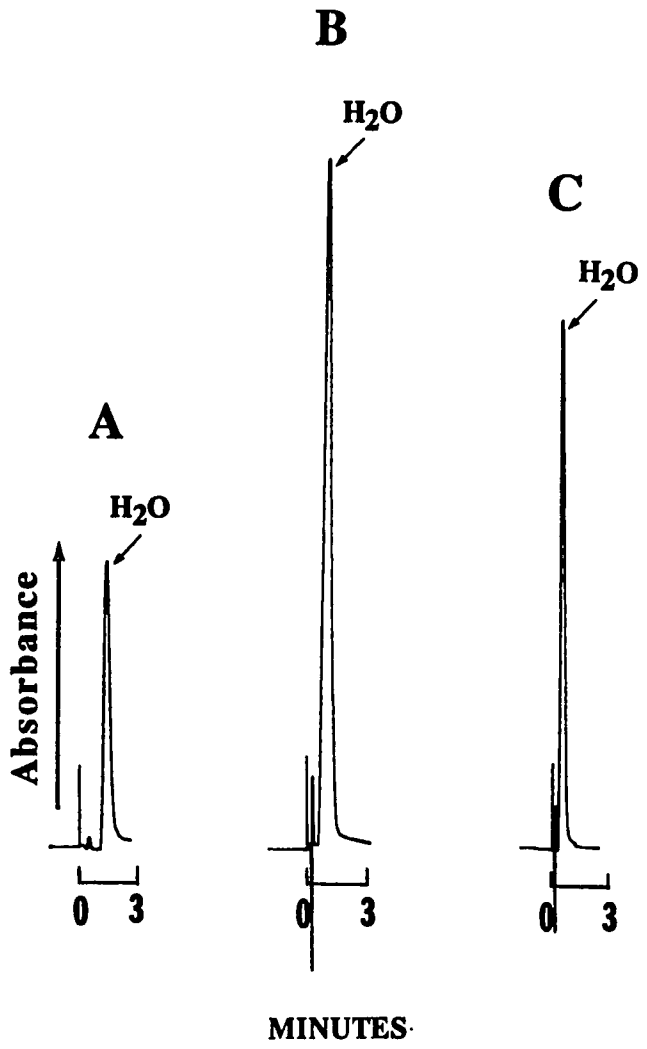


Figure 19. Chromatograms of the same sample obtained using different chromatographic conditions. A, 10 cm x 2.1 mm column, methanol only eluent (plus 1 mM cinnamaldehyde) and 0.5 ml/min flow rate; B, 2.5 cm x 2.1 mm column, 60% methanol and 40% acetonitrile eluent (plus 1 mM cinnamaldehyde) and 0.5 ml/min flow rate; C, 1.0 ml/min flow rate, other conditions are the same as is in B. Sample, 1.0% H_2O in anhydrous acetonitrile

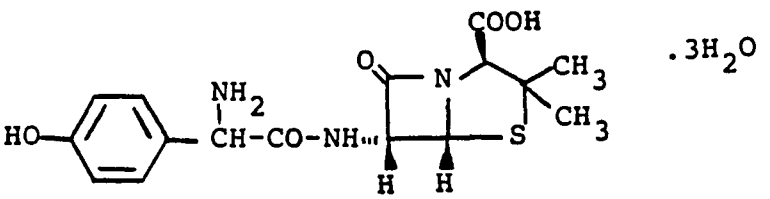
Real Samples

The applicability of the LC method was demonstrated with two pharmaceutical samples, amoxycillin trihydrate and 2-amino-6-chloropurine, which were provided by Beecham Pharmaceutical (Figure 20). The analytical results obtained by that company using different methods varied widely (Table VI). Water in these two samples was determined by our method after dissolving in a 90% methanol and 10% toluene solution containing dilute sulfuric acid. In both cases, the result of the LC method showed good agreement to that of standard KF titration (Table VI). Figure 21 shows that good reproducibility was obtained with the determinations.

Scope of the Method

Successful separations of water in various organic samples were achieved. These samples included aromatic hydrocarbons, unsaturated compounds, chlorinated compounds, alcohols, furans, ethers, and esters. Aldehydes, methyl ketones, tetrahydrofuran (THF), and dimethylsulfoxide (DMSO) gave very broad injection peaks that obscured the water peak. Aldehydes and ketones can undergo an acid-catalyzed reaction with methanol to form acetals, ketals and water, respectively. Interference from DMSO was also noted by Stevens et al. (48). The reason for the

Amoxycillin Trihydrate



2-Amino-6-chloropurine

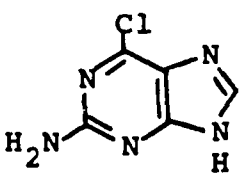


Figure 20. Structures of the two pharmaceutical compounds

Table VI. Determination of water in pharmaceutical compounds with various techniques

Technique	% water found (w/w)	
	Amoxycillin trihydrate	2-Amino-6-chloropurine
Coulometric KF titration[a] (MeOH free reagent)	12.8	1.0
Standard KF titration[a]	13.2	0.9
Weight loss on drying[a] (70 °C, 630 mmHg)	12.6	0.3
Thermogravimetry[a]	12.6	0.2
LC method in this study	13.0, 13.3	0.89, 0.94

[a]Performed by Beecham Pharmaceuticals

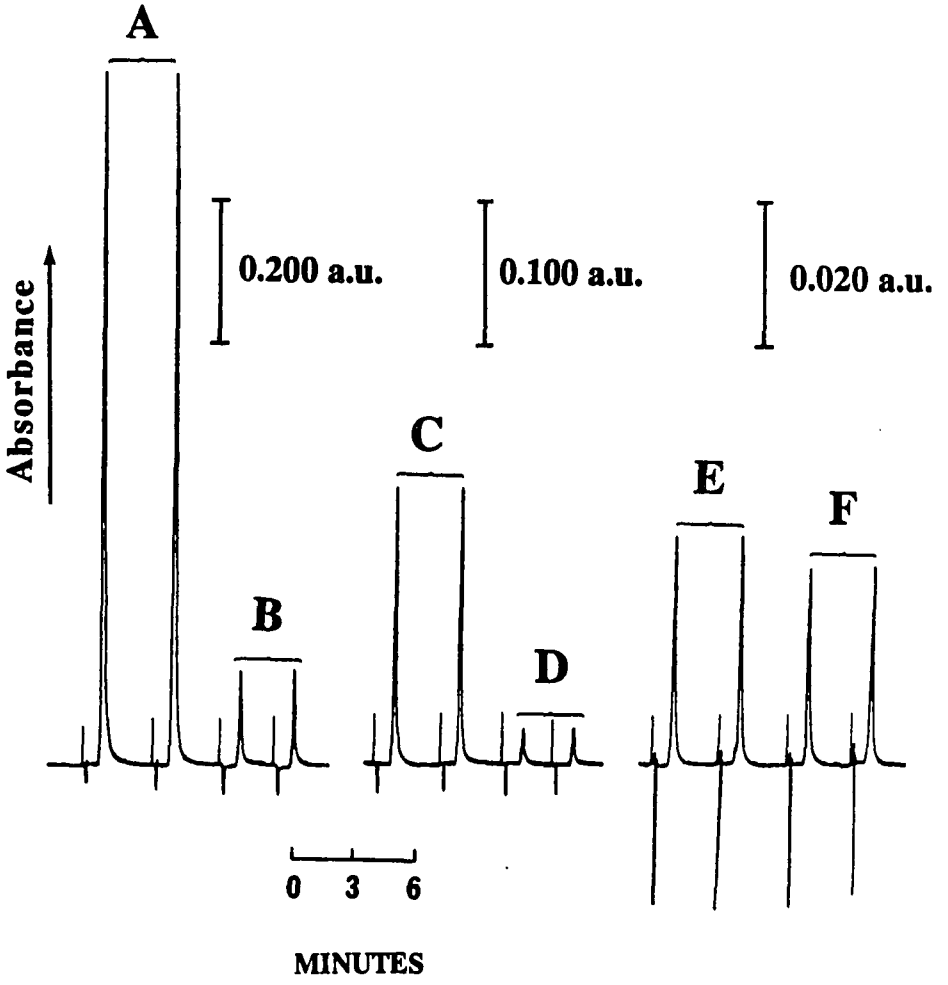


Figure 21. Chromatograms obtained for the determination of water in amoxycillin trihydrates and 2-amino-6-chloropurine. A, 10.0 mg H_2O/ml water standard; B, 1.0 mg H_2O/ml water standard; C, 0.0673 g amoxycillin trihydrates dissolved in 5.00 ml 90% methanol and 10% toluene containing 0.01 M sulfuric acid; 0.0281 g 2-amino-6-chloropurine in 5.00 ml 90% methanol and 10% toluene containing 0.01 M sulfuric acid; D and F, solvent blank. Other conditions are given in the text

interference from THF and DMSO is not clear. Figure 22 shows good chromatograms for water in methanol solution containing acetylcystine and ascorbic acid . These are reducing compounds and cannot be analyzed for water by the Karl Fisher method.

A two-column LC method was developed to determine water in difficult samples encountered by the single-column method and is described in Section II.

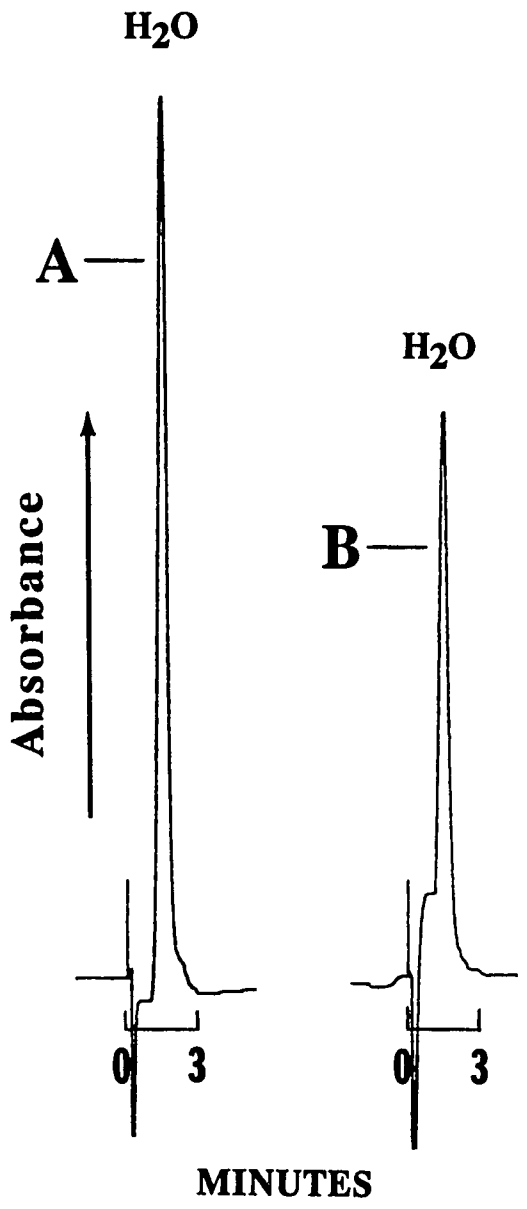


Figure 22. Determination of water in reducing samples. A, 0.155% H_2O in a methanol solution containing 0.083 M ascorbic acid; B, 0.105% H_2O in a methanol solution containing 0.75 M N-acetylcystine. Other conditions are given in the text

CONCLUSIONS

A single-column liquid chromatographic method was developed using a unique spectrophotometric detection system at 300 nm involving an acid-catalyzed cinnamaldehyde-acetal equilibrium. A theoretical model of detection was derived and verified by experiments. A linear calibration curve was obtained which covers more than three orders of magnitude. Detection sensitivity is excellent over a broad concentration range. The lowest concentration of water determined was 26 ppm and the limit of detection is estimated as low as 2 ppm. Reproducibility is excellent with a relative standard deviation of no more than 5%.

Water can be determined within 1 to 2 minutes in a wide variety of samples by ion-exclusion chromatography using only a single separation column. These samples included aromatic hydrocarbons, unsaturated compounds, chlorinated compounds, alcohols, furans, acids, ethers, and esters. Samples containing aldehydes or methyl ketones are subject to interference and require a two-column method described in Section II.

Additional samples were analyzed by the single-column method. The results were listed together in Table V of Section III with those obtained by the gas chromatographic method.

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SECTION II. TWO-COLUMN LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF WATER

INTRODUCTION

In the single-column method discussed in Section I, water in many sample types is separated chromatographically on a single H^+-form cation-exchange column and detected spectrophotometrically using a methanol eluent containing 1 mM cinnamaldehyde. However, aldehydes, methyl ketones react with methanol in the presence of an acid catalyst to form water. For example,

$$CH_3COCH_3 + 2\ CH_3OH \xrightarrow{H^+} (CH_3)_2C(OCH_3)_2 + H_2O \qquad (1)$$

A reaction of this type makes it impossible to separate and determine the water originally present in the sample. Certain other compounds, such as dimethylformamide (DMF) and dimethylsulfoxide (DMSO) (1) also interfere with the single-column method. Fortier and Fritz (2) avoided this difficulty by first separating acetone and water on a neutral cation exchange column (e.g., Li^+-form) in which no reaction occurs between acetone and methanol or between cinnamaldehyde and methanol. This was followed by a cation-exchange column in the H^+ form to catalyze the later reaction and make possible the spectrophotometric detection of the water.

In the present work, this "two-column" method for water is examined

critically. A modified method has been devised that is much faster, more sensitive, and more dependable than the original procedure. The modified method is very broad in scope and appears to have no major interferences.

EXPERIMENTAL SECTION

Apparatus

The chromatographic system consisted of a dual piston LKB 2150 HPLC pump, a model 7010 Rheodyne injector equipped with a sample loop sized 5-μl, a Spectroflow 783 Kratos absorbance detector, and a Curken strip-chart recorder. The columns were packed with a Shandon single-piston packing pump, using upward slurry packing method. Due to the large degree of shrinking and swelling that occurs in polystyrene-divinylbenzene resins when a change in solvent occurs, it was necessary to pack the column in the same solvent used in the mobile phase.

Reagents

Trans-cinnamaldehyde (99%) and anhydrous acetonitrile were purchased from Aldrich Chemicals (Rochester, NY) and were used without further purification. Karl Fischer grade (anhydrous) methanol, one-component reagent for Karl Fischer titration (HYDRANAL-Composite 2, 1 ml = 2 mg H_2O), and water standards (1.00 ± 0.02 mg H_2O and 5.00 ± 0.02 mg H_2O per ml) were obtained from Fisher Scientific (Pittsburgh, PA). Peroxides were purchased from Fluka Chemie AG. Aminex Q-150s, Aminex 50W-X4, and Aminex A-7 cation exchange resins in Na^+ form were from Bio-Rad

(Richmond, California). All other chemicals were reagent grade or better and were used without purification. Distilled water was further purified with the Barnstead Nanopure II System before use.

Eluent and Standard Samples

Eluent was prepared simply by dissolving carefully weighed amount of cinnamaldehyde to anhydrous methanol and acetonitrile used as mobile phase. Standard samples were prepared by adding accurately measured volumes of water to known volumes of anhydrous acetonitrile or acetone contained in vials equipped with hole caps and Teflon-faced Neoprene septa (Supelco Inc, Bellefonte, PA). For maximum sensitivity and reproducibility, the eluent and all standard samples were prepared under the protection of dried nitrogen. Once prepared, the eluent was protected from atmospheric moisture using a septum capped reservoir and a balloon filled with dry nitrogen which was connected to the reservoir through a needle.

Columns

For the two-column method, the separation column was a 15 cm x 4.6 mm stainless steel column packed with Aminex Q-150S resin in Li^+ form, and the catalyst column was a 2.5 cm x 2.1 mm stainless steel column

packed with the Aminex Q-150S resin in H^+ form. The Aminex Q-150S resin as received in the Na^+ form was converted to Li^+ or H^+ form by equilibrating with a methanol solution containing either 0.5 M $LiClO_4$ or 1.0 M H_2SO_4. The packed columns in Li^+ and H^+ form were further washed with solutions of 0.5M $LiClO_4$ and 0.1 M H_2SO_4 respectively, at a flow rate of 1 ml/min for 2 hours before use. Small amount of LiOH was added to the lithium salt solution to neutralize the trace acid contained in the salt.

Chromatographic Conditions

Unless specified, the following chromatographic conditions were employed for the entire experimental work: A sample loop sized 5-μl, a flow rate at 1.2 ml/min, an eluent of 1 mM cinnamaldehyde dissolved in 40% methanol and 60% acetonitrile, and a detection wavelength at 300 nm.

Calibration

The water peak heights were determined for a series of standard samples and plotted against the added amounts of water. Two water standards (1.00 mg H_2O/ml and 5.00 mg H_2O/ml) were used to standardize the calibration curve. The difference in water peak height between the acetonitrile standard containing 0.50% added water and the water

standard (5.00 mg H_2O/ml) was used to determine the water concentration in the sample matrix (acetonitrile).

RESULTS AND DISCUSSION

Optimization of Experimental Variables

Starting with the conditions recommended in Section I, the dimensions of the columns, cation form of the resin, eluent composition, flow rate, and detection wavelength were varied systematically in order to find the best chromatographic conditions. The determination of water in acetone was used in these experiments because it poses one of the most difficult separation problems.

Column dimensions

A 15 cm x 4.6 mm stainless steel separation column packed with Li^+-form cation exchange resin, followed by a 2.5 cm x 2.1 mm stainless steel column containing H^+-form cation exchange resin (Figure 1) was found to give an excellent separation of the acetone and water peaks. The separation column is smaller in diameter and contains less resin than that used in an earlier paper (2). Likewise, a very small catalytic column (2.5 cm x 2.1 mm) is entirely adequate. At similar flow rates the retention time of the water peak is now 3 to 4 minutes compared with about 13 minutes using the earlier column system. A separation column of 2.1 mm i.d. was found inadequate to separate the

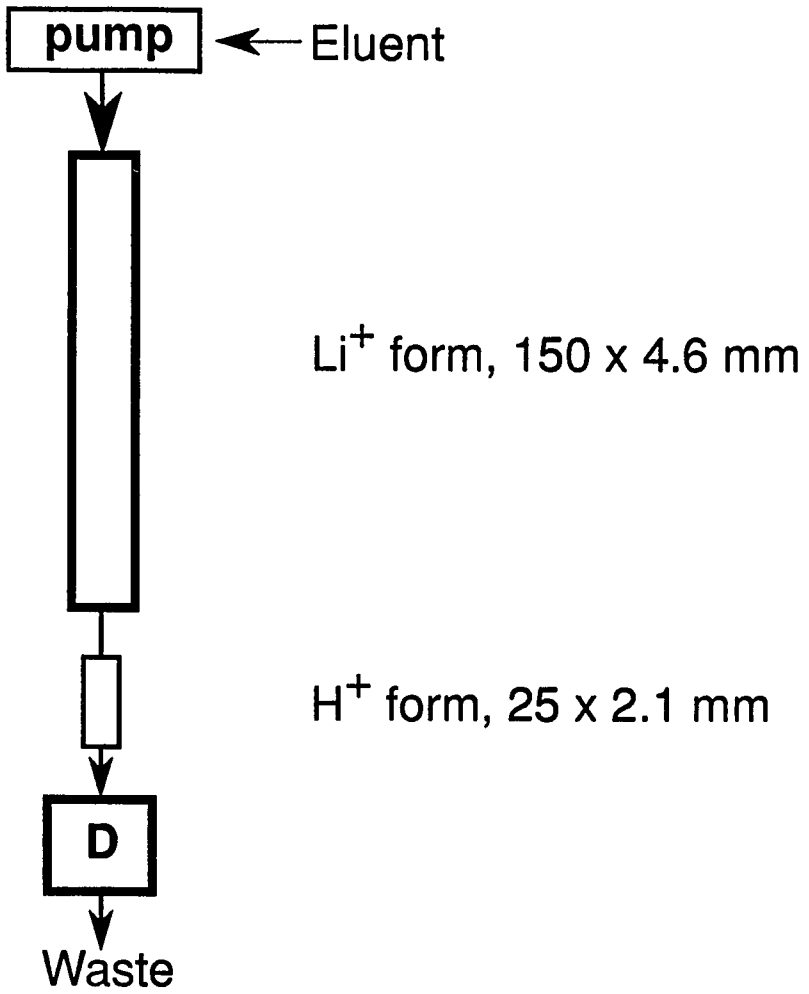

Figure 1. Schematic diagram of the two-column configuration

water and the aldehydes or ketones peaks.

Ionic from of the resin

Various separation columns packed with resins in different ionic forms were compared using a constant amount of water added to an acetonitrile sample (Figure 2). In each case a 25 x 2.1 mm catalytic column in the H^+ form was placed in line between the separation column and the detector cell. The following conclusions can be drawn regarding the ionic form of the separation column. Retention time of water: H^+ >> Na^+ and Li^+; Peak width: Na^+ >> H^+ > Li^+; Sensitivity: Li^+ >> H^+ > Na^+. The much longer retention time obtained with the H^+ form resin is believed to be caused by hydrogen bonding interaction besides the ion-exclusion mechanism (3). Resins in Li^+ and Na^+ form gave similar retention time but the later produced a much broader peak. These is probably due to the fact that the radius of Na^+ is larger than Li^+, which reduces the actual size of the pore in the micro porous Aminex Q-150S resin (4). The reduced pore size prevents the water molecules from travelling freely into and out from the inside of the resin and results in a broad water peak. The sensitivity is also affected by the peak shape because peak height instead of peak area was used as the signal.

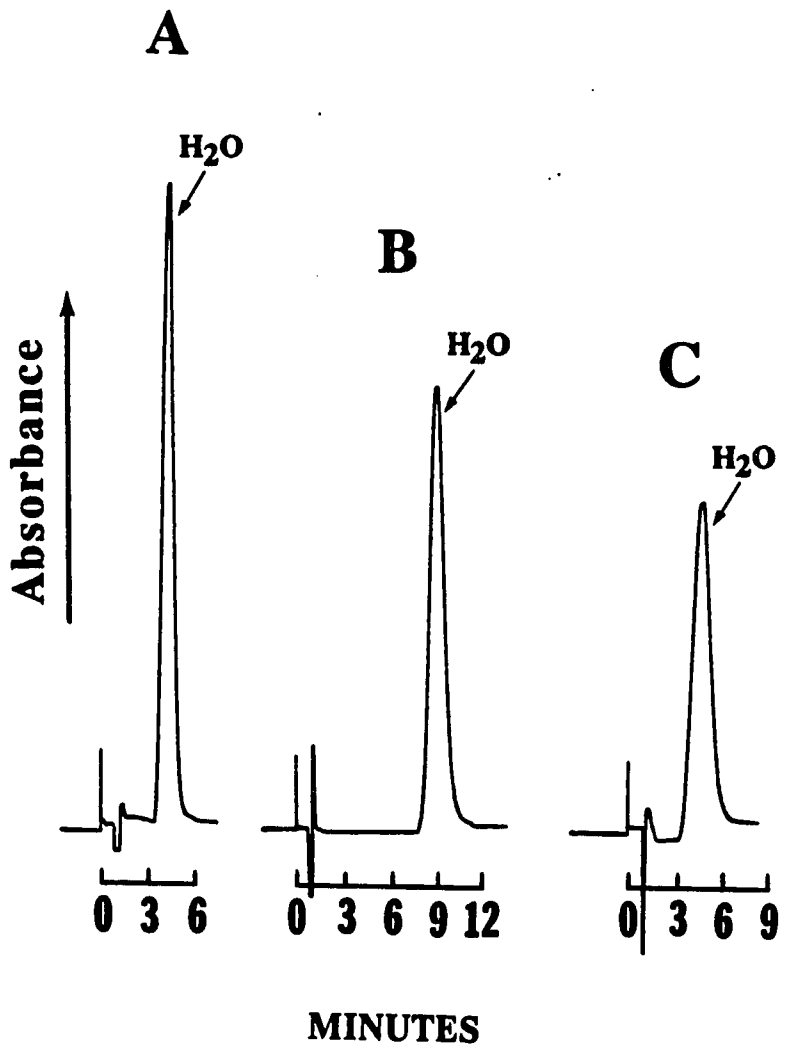

Figure 2. Chromatograms of the same sample obtained with separation columns in various ionic forms. A, Li^+-form resin; B; H^+-form resin; C, Na^+-form resin. Sample, 1.0% H_2O in anhydrous acetonitrile. Other conditions are given in the text

Eluent composition, flow rate, and detection wavelength

The composition of the eluent was varied from 100% methanol to 20% methanol and 80% acetonitrile. Trends similar to those described in Section I were found (Figure 3). Again, sensitivity increased with increasing proportions of acetonitrile in the eluent. An eluent containing 40% methanol and 60% acetonitrile was used for all the subsequent experiments. Eluent containing 20% methanol and 80% acetonitrile gave a higher sensitivity, but also a rather unsteady baseline. As would be expected, a faster flow rate decreases the retention time but also lowers the sensitivity (Figure 4). A flow rate of 1.2 ml/min is employed for most separations on a 15 cm x 4.6 mm Li^+ form column. At this flow rate, the retention time of the water is now reduced to about 4 minutes instead of 13 minutes in the previous method (2). A detection wavelength of 300 nm was again found to be somewhat superior to the wavelength of 310 nm previously recommended (2).

Determination of Water in Aldehydes and Ketones

The injection peak of an acetone sample is caused by the water formed by the reaction of acetone with methanol from the eluent in the catalyst column. Some difficulty was encountered initially in obtaining a satisfactory separation of the water and acetone peaks using the

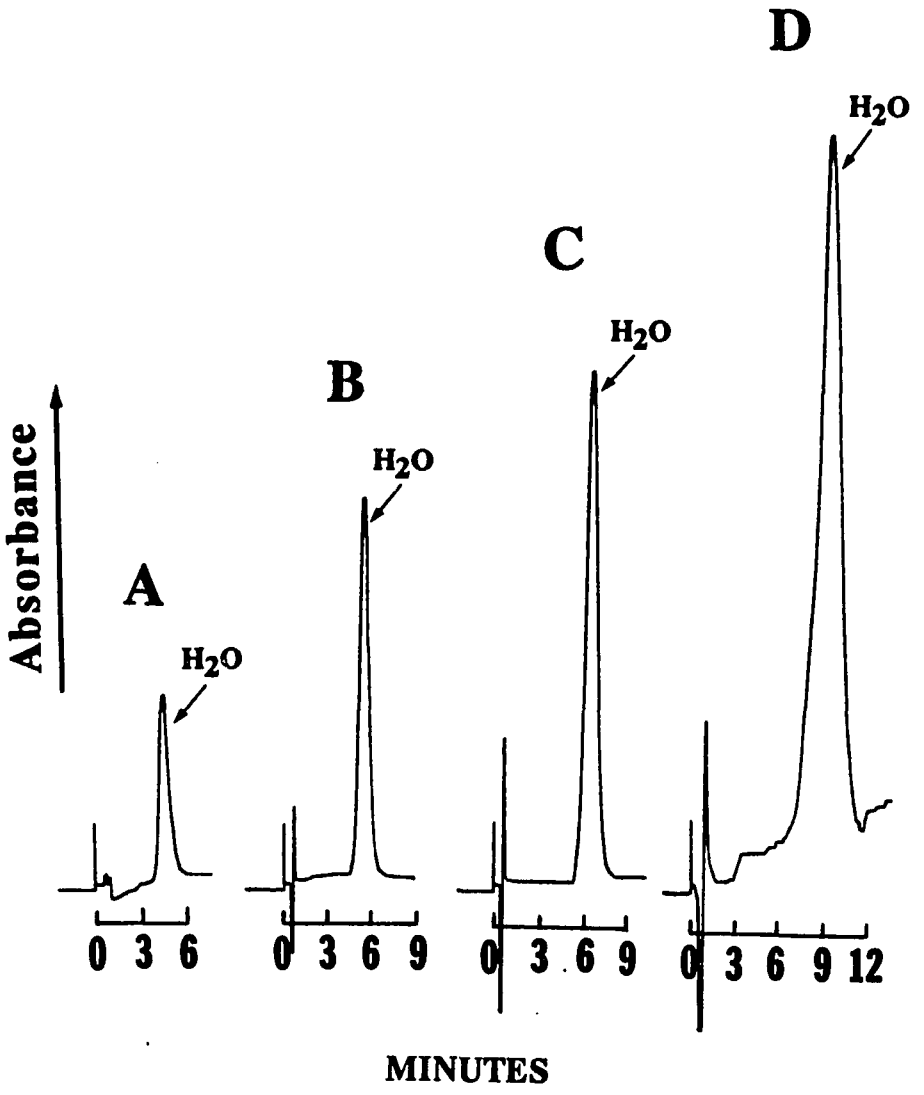

Figure 3. Chromatograms of the same sample obtained with eluents containing different proportions of acetonitrile. A, 100% methanol; B, 60% methanol and 40% acetonitrile; C, 40% methanol and 60% acetonitrile; D, 20% methanol and 80% acetonitrile. Sample, 1.0% H_2O in anhydrous acetonitrile. Other conditions are given in the text

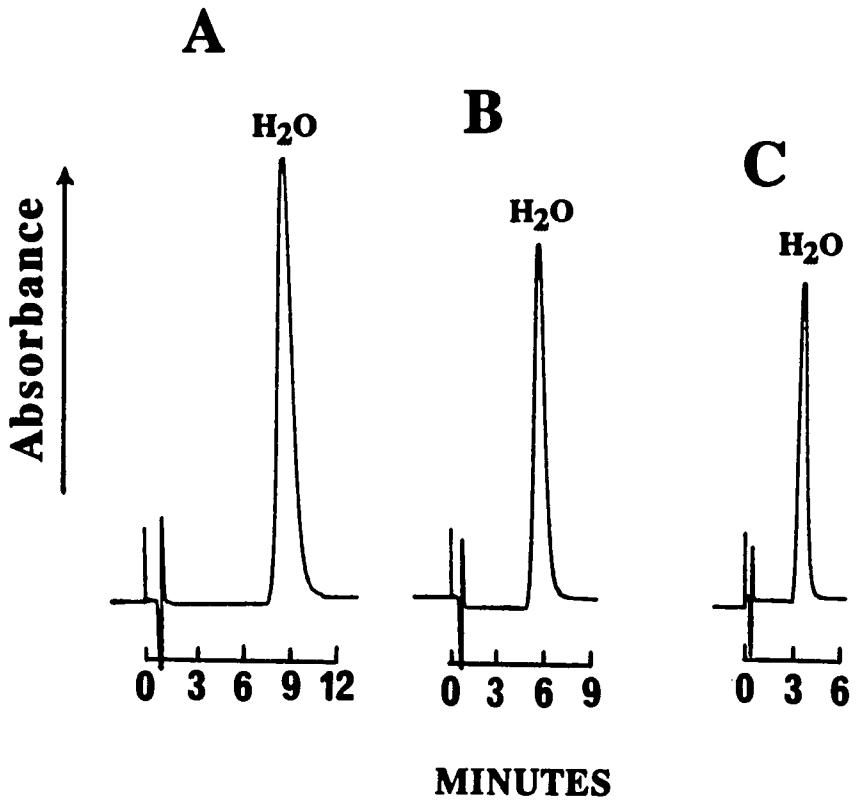

Figure 4. Chromatograms of the same sample obtained with different flow rates. A, 1.2 ml/min; B, 2.0 ml/min; C, 3.0 ml/min. A 15 cm x 4.6 mm separation column in H^+ form was used. Sample, 1.0% H_2O in anhydrous acetonitrile. Other conditions are given in the text

Li^+-form separation column. For example, washing with a lithium perchlorate solution in methanol to ensure complete conversion of the cation exchanger to the Li^+ form gave an incomplete separation (Figure 5b) and eventually no separation at all (Figure 5c). The difficulty was traced to H^+ impurities in the lithium perchlorate that prematurely catalyzed the reaction of acetone with methanol to form water (Equation 1). After washing the separation column with a little methanol solution containing 0.1 M lithium hydroxide to neutralize the H^+, an excellent separation was obtained (Figure 5a).

Water can easily be determined in aldehydes and ketones so long as the Li^+-form separation column does not contain any H^+. Chromatographic separations of water in four different aldehydes are shown in Figure 6. Water in two ketones was also nicely determined as is shown in Figure 7.

Determination of Water in Peroxides

Although it is frequently necessary to determine the amount of water in various peroxides and peroxide solutions, this is not an easy analysis to perform. The oxidizing properties prevent the use of a Karl Fischer titration (5).

It is possible to determine water in peroxides and hydroperoxides by

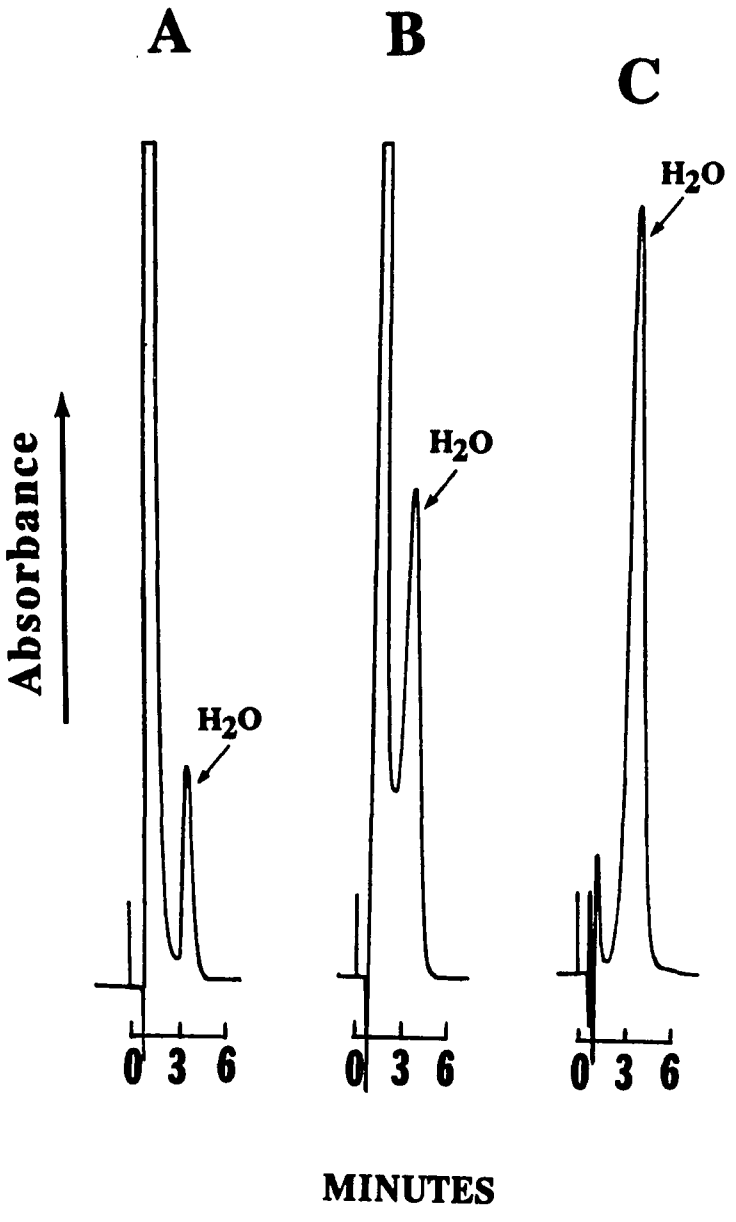

Figure 5. Chromatograms of the same acetone sample obtained with separation columns treated with different solutions. A, column washed with 0.5 M $LiClO_4$ in methanol that also contains a small amount of LiOH; B, column washed with 0.5 M $LiClO_4$ in methanol for 1 hr.; C, column washed with 0.5 M $LiClO_4$ in methanol for 3 hrs. Other conditions are given in the text

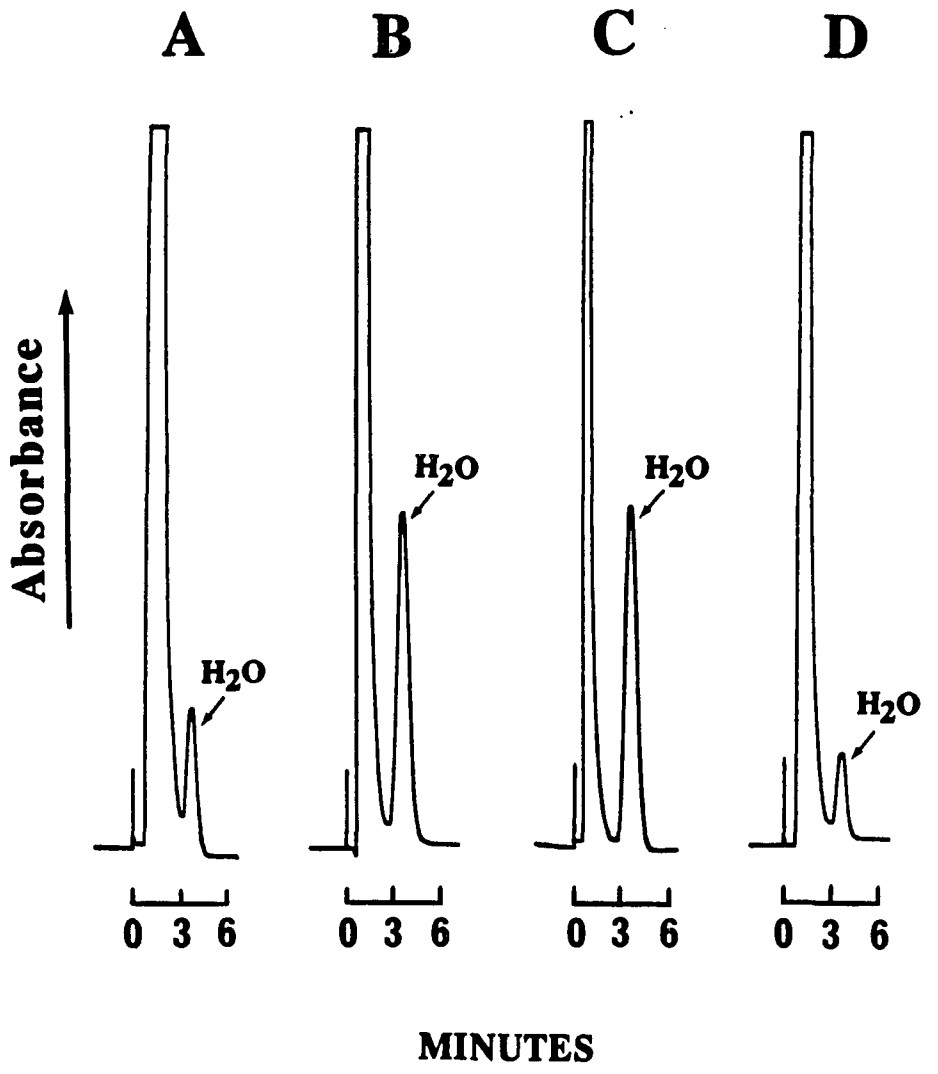

Figure 6. Determination of water in various aldehydes. A, 0.11% water in acetaldehyde; B, 1.57% water in propionaldehyde; C, 0.81% water in heptaldehyde; D, 0.19% water in octylaldehyde. Other conditions are given in the text

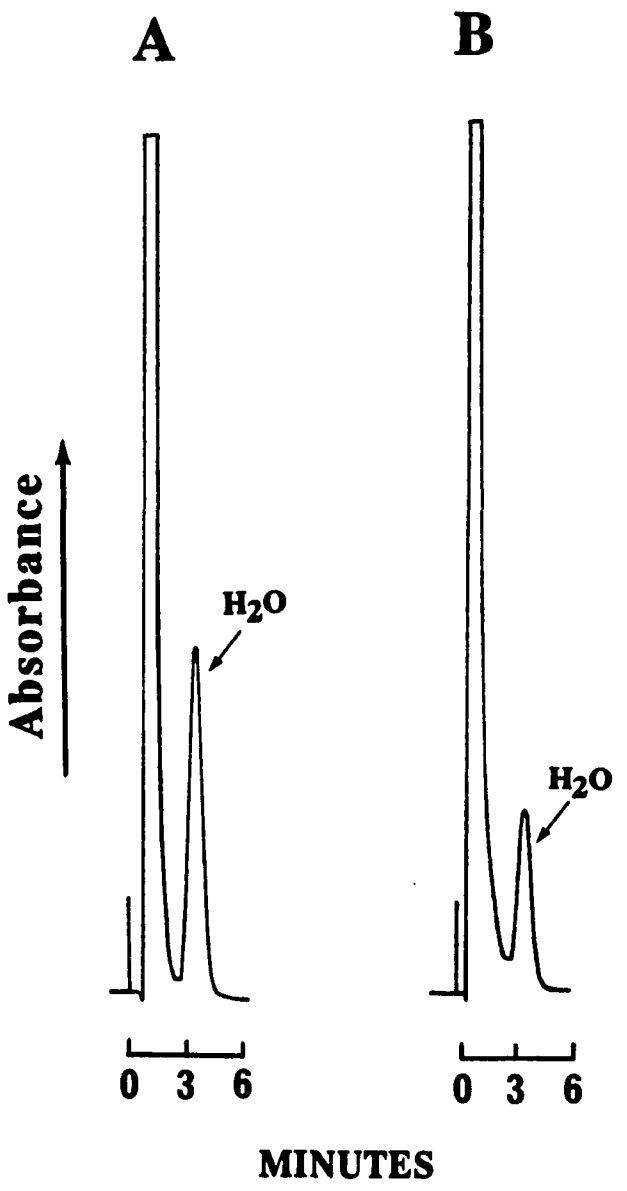

Figure 7. Determination of water in various ketones. A, 1.41% H_2O in acetone; B, 0.39% H_2O in 2-methyl-3-octanone. Other conditions are given in the text

the two-column chromatographic method. The peroxide and water are separated under neutral conditions on the Li^+-form column, so that there is no interference in the reaction of water with the cinnamaldehyde-acetal detection system in the catalytic column. Chromatograms for determination of water in three different peroxides are shown in Figure 8.

Determination of Water in Other Compounds

Figure 9 shows chromatograms for the determination of water in dimethylsulfoxide (DMSO), dimethylformamide (DMF), 3-mercaptopropionic acid and acetic acid. It is not possible to determine water in these samples by the single-column method (2,6) or in the mercaptan by the Karl Fischer method (5).

Table I lists the organic samples that have been successfully analyzed for water, together with the water content found by chromatographic analysis.

Quantitation

Several calibration curves were prepared using anhydrous acetonitrile and acetone to which carefully measured amounts of water had been added. The calibration plots were always linear. The

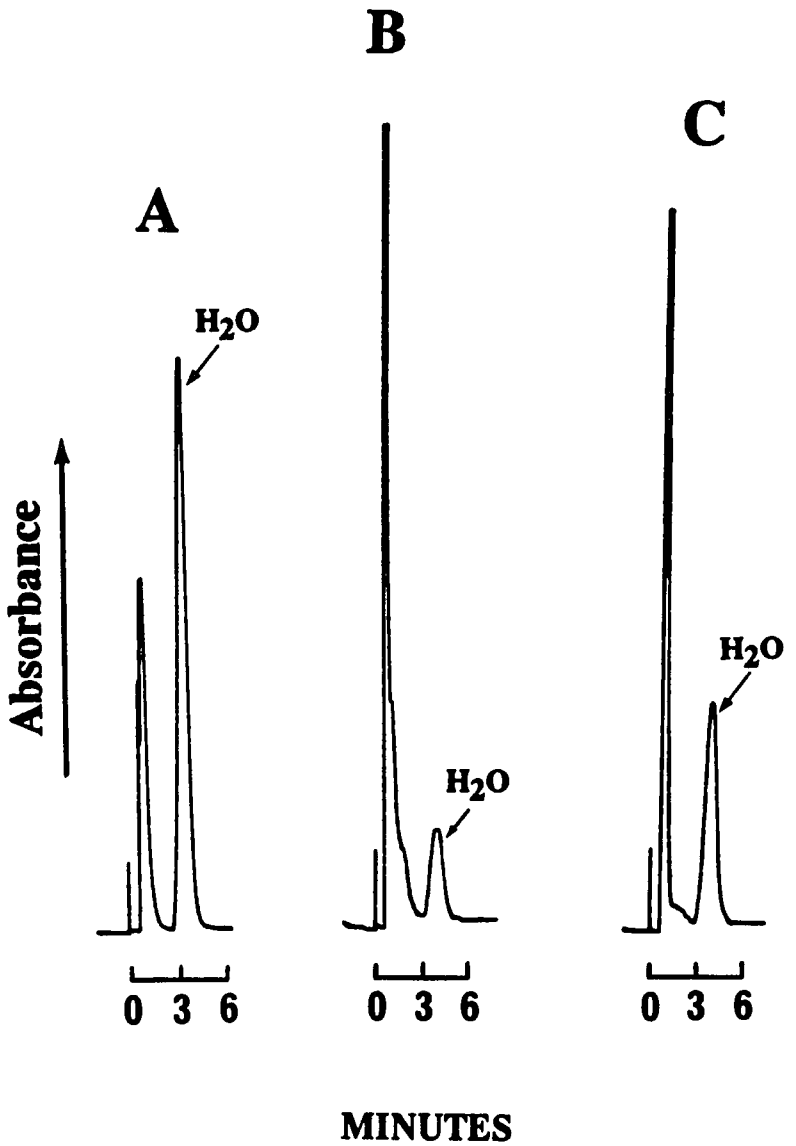

Figure 8. Determination of water in various peroxides. A, 8.4% water in 2-butanone peroxide; B, 0.10% water in tert-butyl peroxide; C, 0.37% water in a toluene solution containing 5% benzoyl peroxide. Other conditions are given in the text

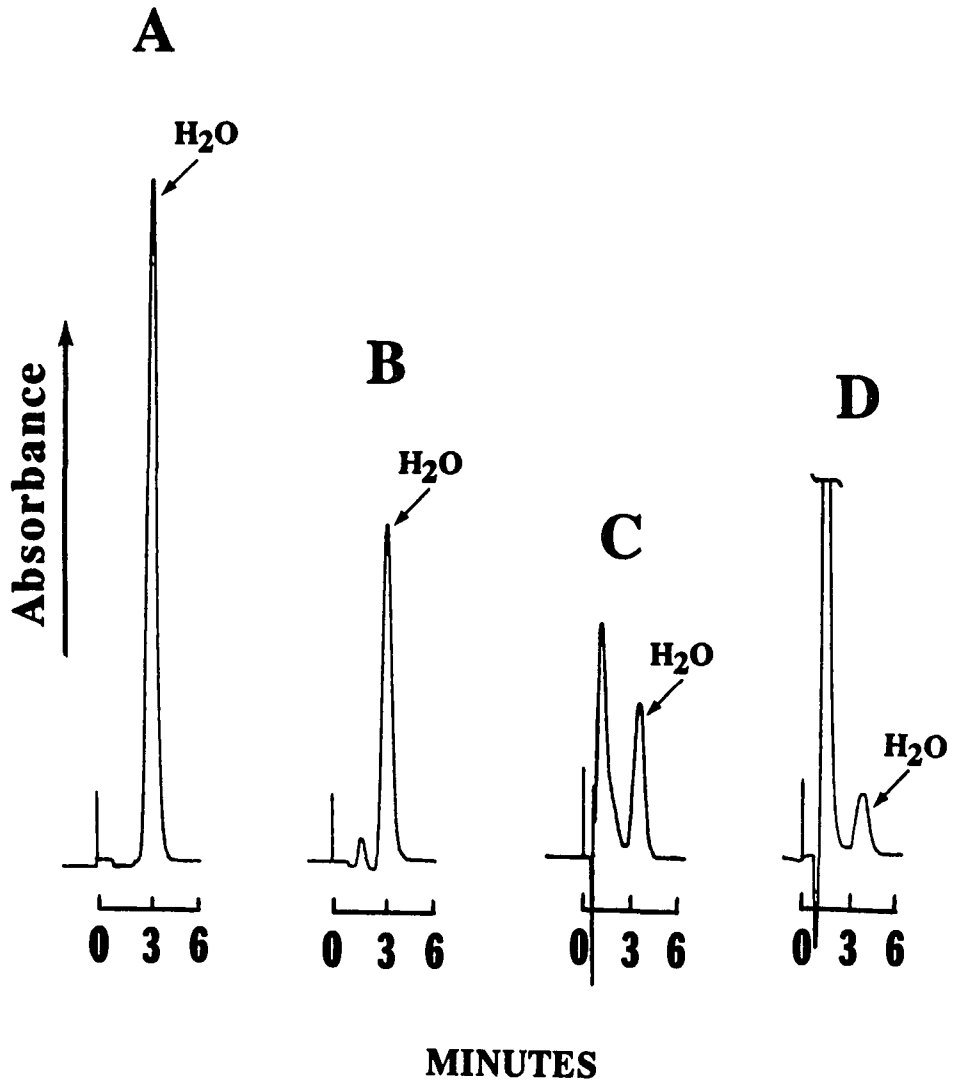

Figure 9. Determination of water in various samples. A, 9.61% water in DMSO; B, 4.84% water in dimethylformamide; C, 0.72% water in 3-mercaptopropionic acid; D, 0.13% water in glacial acetic acid. Other conditions are given in the text

Table I. Compounds analyzed for water using two-column method

Compound	$\%H_2O$ (v/v)
Acetaldehyde	0.11
Propionaldehyde	1.57
Heptaldehyde	0.81
Octylaldehyde	0.19
Acetone	0.38
2-Methyl-3-octanone	0.29
2-Butanone peroxide	8.42
tert-Butyl peroxide	0.10
5% Benzoyl peroxide in Toluene	0.37
Acetic acid (glacial)	0.13
Lactic acid	0.17
3-mercaptopropionic acid	0.72
Acetic anhydride	0.066
Dimethylformamide	4.84
Tetrahydrofuran	0.79
Ethyl acetate	0.67
Ethyl ether	1.2
Methylene chloride	0.083
2-Propylnol	0.40

correlation coefficient for the acetonitrile standards was 0.99999 in the range of 0.02% to 4.0%, 0.9995 in the range of 0.02% to 20.0% water, and the slope was 0.060 a.u./1% water. The slope and correlation coefficient for the acetone standards were essentially the same.

The sensitivity (slope of the calibration curve) obtained with the two-column method is about 17 times less than that by the single-column method. This is mainly because that the water peak is significantly broadened by the much longer and larger Li^+-form separation column. However, this sensitivity is still several times greater than that in the previous method (2). An even higher sensitivity is expected if peak area instead of peak height is used as the detector signal.

CONCLUSIONS

Water is separated from the sample matrix on a neutral Li^+-form column, which is followed by a short H^+-form column to catalyze the chemical reaction needed for detection of water. The two-column method is refined so that water can be determined quickly and accurately in almost any kind of organic sample, including aldehydes, ketones, carboxylic acids, and peroxides. Experimental variables have been carefully studied in order to optimize this two-column method. Good sensitivity is also obtained with the method.

The single-column method described in Section I, complemented by the two-column method, is applicable to a wide variety of samples. No interference was encountered in the two-column method for water other than amines, which react with the H^+ in the catalytic column and reduces the catalytic activity of the column.

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SECTION III. GAS CHROMATOGRAPHIC DETERMINATION OF WATER AFTER REACTION WITH TRIMETHYLORTHOFORMATE

INTRODUCTION

The problem of determining the amount of water in analytical samples is so widespread that a great many approaches have been used. In addition to the classical Karl Fischer titration (1), a number of methods have been developed that use liquid chromatography (2-6) or gas chromatography (7-10). The merits and limitations of these methods have been discussed in the previous papers from this group (4-6, 10).

Recently, Dix, Sakkinen, and Fritz (10) published a method in which water reacts with 2,2-dimethoxypropane (DMP) in the presence of a solid acid catalyst. A product of the reaction (acetone) is then determined by capillary-column GC using a flame-ionization detector. Although this method is reliable and broad in scope, several drawbacks associated with the use of DMP and a solid acid catalyst limit the general usefulness of the method. The reaction rate is relatively slow due to the heterogeneous nature of the solid acid catalyst. As a result, the reaction requires at least five minutes constant shaking to reach completion. Because of the relatively small equilibrium constant, the completeness of the reaction between water and DMP is not acceptable when water content in a sample is low. In fact, negative water contents are obtained for samples containing actual concentration of water in the

low parts per million level. Therefore, it would be advantageous to use a reagent which reacts more completely with water.

Ortho esters are known to react with water under acidic conditions to form a carboxylic acid ester plus an alcohol. The mechanism and kinetics of this reaction have been studied extensively (11-14). However, to the authors' knowledge no method for determining water based on this reaction has been reported. We have found that a liquid acid catalyst can be dissolved in the ortho ester reagent and thus be added to the sample together with the reagent. The reaction is almost instantaneous and quantitative even when water is present at trace level. The acid catalyst does not damage the capillary GC column used to determine the concentration of one of the reaction products.

In this report, a rapid, sensitive method based on the reaction of water with an ortho ester is described.

EXPERIMENTAL SECTION

Reagents and Chemicals

The ortho esters, 2,2-dimethoxypropane, 3-methylpentane, methanesulfonic acid, and anhydrous solvents were purchased from Aldrich Chemicals (Rochester, NY). The one-component reagent for Karl Fischer titration (HYDRANAL-Composite 2, 1 ml = 2 mg H_2O) and water standards (5.00 ± 0.02 mg H_2O/ml and 1.00 ± 0.02 mg H_2O/ml) were obtained from Fisher Scientific (Pittsburgh, PA). Standard samples were prepared by adding measured volumes of water to known volumes of anhydrous N,N-dimethylformamide. All other reagents were of reagent grade or better. Distilled water was further purified with the Barnstead Nanopure II system before use.

Gas Chromatography

A Hewlett-Packard 5880A gas chromatograph equipped with a flame ionization detector (FID) and a Hewlett-Packard 7673A automatic sampler was used in the split injection mode. The split ratio was about 100:1 and was held constant during the experiments. The column was a 30 m x 0.53 mm i.d. J&W DB-5 Megabore with a film thickness of 1.5 μm. A split glass liner (4-mm i.d., Hewlett-Packard) packed with 0.3 g Chromosorb W-

HP coated with 3% silicone OV-1 (80-100 mesh, Alltech Associates) was placed in front of the column to prevent any nonvolatile residue from entering the column. Both the injector and detector temperatures were held at 250 °C. An oven temperature between 40°C and 110°C was chosen, depending on the reagent used. An injection volume of 1 μl was used through out the entire experiment. Isothermal elution was employed for most samples. The column was cleaned periodically by stepping the oven temperature to 250°C and maintaining this temperature for a period of time. Zero grade helium was used as the carrier gas.

Reactant Solution

For the analysis, a reactant solution was prepared by mixing 10.0 ml of ortho ester or DMP (reagent), 1.0 ml of 3-methylpentane (internal standard), and 7.1 μl (10 mM) of methanesulfonic acid (catalyst) in a 30-ml bottle equipped with screw hole cap and Teflon-faced Neoprene septum obtained from Supelco (Bellefonte, PA). This solution permits a simple one-step addition of all the necessary chemicals and catalyst. The hole capped bottle protects the reactant solution from the atmospheric moisture and yet allows convenient transfer of the reactant solution with air-tight syringes (Hewlett Packard, Avondale, PA). For systematic studies and comparison experiments, other acid catalysts such

as hydrochloric acid and sulfuric acid were employed instead of methanesulfonic acid.

Procedure

1. Inject 1 μl of reactant solution into the GC and chromatograph using the conditions described under Gas Chromatography. Measure the response of the ethanol peak relative to that of the internal standard peak in order to determine the water blank.

2. Prepare a calibration plot as follows. Add 0.50 ml of a standard sample of known water content and 1.00 ml of reactant solution to a 2-ml sample vial equipped with a crimp cap and a Teflon-lined septum (Hewlett Packard, Avondale, PA). Shake the mixture briefly and inject 1 μl into the GC as in Step 1. Measure the response of the ethanol peak relative to the internal standard peak. Subtract the relative response of the water blank from this in order to obtain the corrected relative response. Repeat this measurement for several standard samples. Prepare a linear plot of corrected relative response vs. water concentration and measure the slope.

3. Determine the concentration of water in actual samples as follows. Measure the corrected relative response of a 0.50 ml sample under exactly the same conditions as the water standards in Step 2.

Calculate the water concentration by dividing the corrected relative response by the slope of the calibration plot.

Better accuracy and reproducibility were obtained on samples of low water content by using a smaller volume of reagent solution. For example, 0.050 ml of reagent solution was suitable for 0.50 ml samples containing 1% or less water. However, the calibration curve must be run with exactly the same volumes of reagent and sample as the sample.

Solid samples were dissolved in an appropriate solvent such as methanol or N,N-dimethylformamide before analysis. Samples containing an organic base were neutralized with a 0.1 M solution of sulfuric acid in methanol before mixing with the reactant solution. The solid salt formed was separated from the solution by centrifuging.

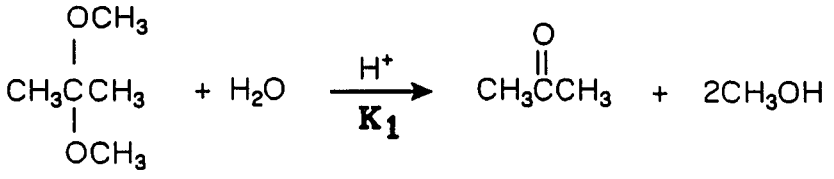
Karl Fischer Titration

Karl Fischer titration was performed with a home made closed system consisting of a 10-ml semi-automatic buret, a 150-ml Erlenmeyer flask and a small magnetic stir bar. The system was protected from moisture using drying tubes filled with drierite. The one-component reagent obtained from Fisher Scientific was standardized using either water standards or deionized water. A visual end-point was employed.

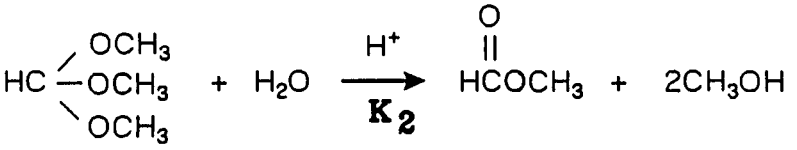
RESULTS AND DISCUSSION

Comparison of an Ortho Ester and DMP

The reaction of an ortho ester such as trimethylorthoformate (TMOF) with water (Equation 2) is similar to the reaction of the dimethyl ketal of acetone with water (Equation 1).



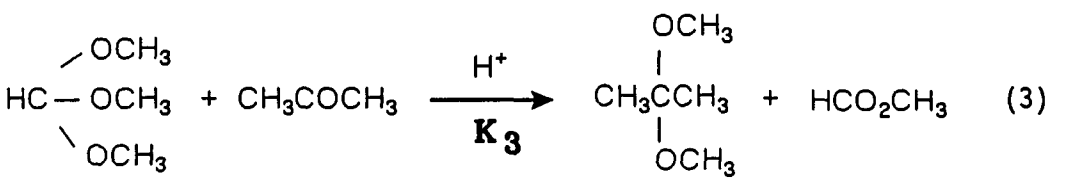
(1)



(2)

The equilibrium constant for Equation 1 (K_1) has been reported to be 2.5 x 10^3 mole l^{-1} (15, 16). This means that the reaction with water may be less than quantitative when water content of a sample is low. The equilibrium constant for Equation 2 (K_2) is not available from literature. Our attempt to determine K_2 failed owing to the difficulty in quantifying the extremely low equilibrium concentration of TMOF by

GC. Although K_2 remains unknown, our experiments indicate that the reaction of TMOF with water is more complete than that of DMP. This may also be deduced from the fact that DMP is produced from the reaction of acetone with TMOF (Equation 3).



(3)

Equation 3 represents a popular synthetic route for preparing ketals from ketones and can be obtained by subtracting Equation 1 from Equation 2. We know that this reaction lies far to the right and therefore K_3 ($K_3 = K_2/K_1$) should be much greater than one. This is also equivalent to saying that K_2 is much greater than K_1.

The superiority of an ortho ester over DMP for reaction with water was demonstrated by analyzing several samples of low water content using each reagent. The results in Table I show negative water contents for three of the samples analyzed by the DMP method. The fourth sample gives positive but incorrect result. Analysis of the same samples using an ortho ester, triethylorthoformate (TEOF), give higher results which are in agreement with those obtained by the Karl Fischer titration method.

Table I. Water contents obtained for several anhydrous organic solvents using DMP or TEOF as the reagent

Sample	Water content (ppm) (n = 2)		
	DMP	TEOF	KF titration
Cyclohexane	-21.7	13.4	14.3
Ethyl ether[a]	-17.5	19.8	
Tetrahydrofuran[a]	-16.0	16.8	
Benzene[b]	31.2	60.6	59.5

[a]Distilled after overnight refluxing with sodium and benzyl alcohol

[b]Distilled after overnight refluxing with lithium aluminum hydride

Choice of Ortho Ester

Several ortho esters were tried for the analytical determination of water based on the acid-catalyzed reaction (Equation 2), followed by the GC determination of the corresponding ester or the alcohol. These included TMOF, TEOF, trimethylorthoacetate (TMOA), triethylorthoacetate (TEOA), and triethylorthopropionate (TEOP). All of these ortho esters

are liquids and can be mixed with an acid catalyst and added to a liquid sample without adding any additional solvent. The orthoformate esters were found to give the most rapid and complete reactions. TEOF was selected for all subsequent work because the reaction products (ethyl formate and ethanol) gave a stronger FID detection signal than the corresponding reaction products of TMOF. Also a higher oven temperature (80°C) was employed for TEOF, which was desirable for the elution of high boiling sample matrices.

Selection of Acid Catalyst

Preliminary experiments showed that a low concentration of a strong acid in the liquid ortho ester was sufficient to catalyze the reaction with water. The ability to use a homogeneous acid catalyst is a great convenience over our previous method in which a solid acid catalyst had to be measured out for each determination and the reaction mixture shaken for at least 5 minutes before analysis by GC (10). The general requirements of a suitable acid catalyst are as follows: low water content, good solubility in the reagent, strong acid strength so that only a low concentration is needed, and sufficient volatility to prevent build up in the gas chromatograph.

Various inorganic and organic acids were tested in order to find a

catalyst that best meets these general requirements. Of the acid catalysts tried, acetic acid, dichloroacetic acid and trifluoroacetic acid required an excessively high concentration (> 0.1 M) for effective catalysis. Hydrochloric acid (37%) and hydrogen chloride in ethyl ether or acetic acid worked well but had a high water background. Trifluoromethane sulfonic acid was too hydroscopic. Concentrated sulfuric acid had a rather low solubility in the ortho ester reagent. The best acid catalyst was methanesulfonic acid (99%). Because a very low concentration was used, no deterioration of the capillary GC column resulted from extended use of this acid catalyst.

Minimum Acid Concentration Required

Varying concentrations of methanesulfonic acid were added to several reactant solutions of the ortho esters and DMP in order to determine the acid concentration needed for a reaction with water to complete within certain time period. This was done by measuring the percent reaction vs. reaction time for a given acid concentration. An example of such a measurement is given in Figure 1. The minimum acid concentrations required for a reaction of an ortho ester that was completed within 1 minute are summarized in Table II. Table II shows that a concentration of only 0.5 mM is needed for the TEOF reagent. This means that the

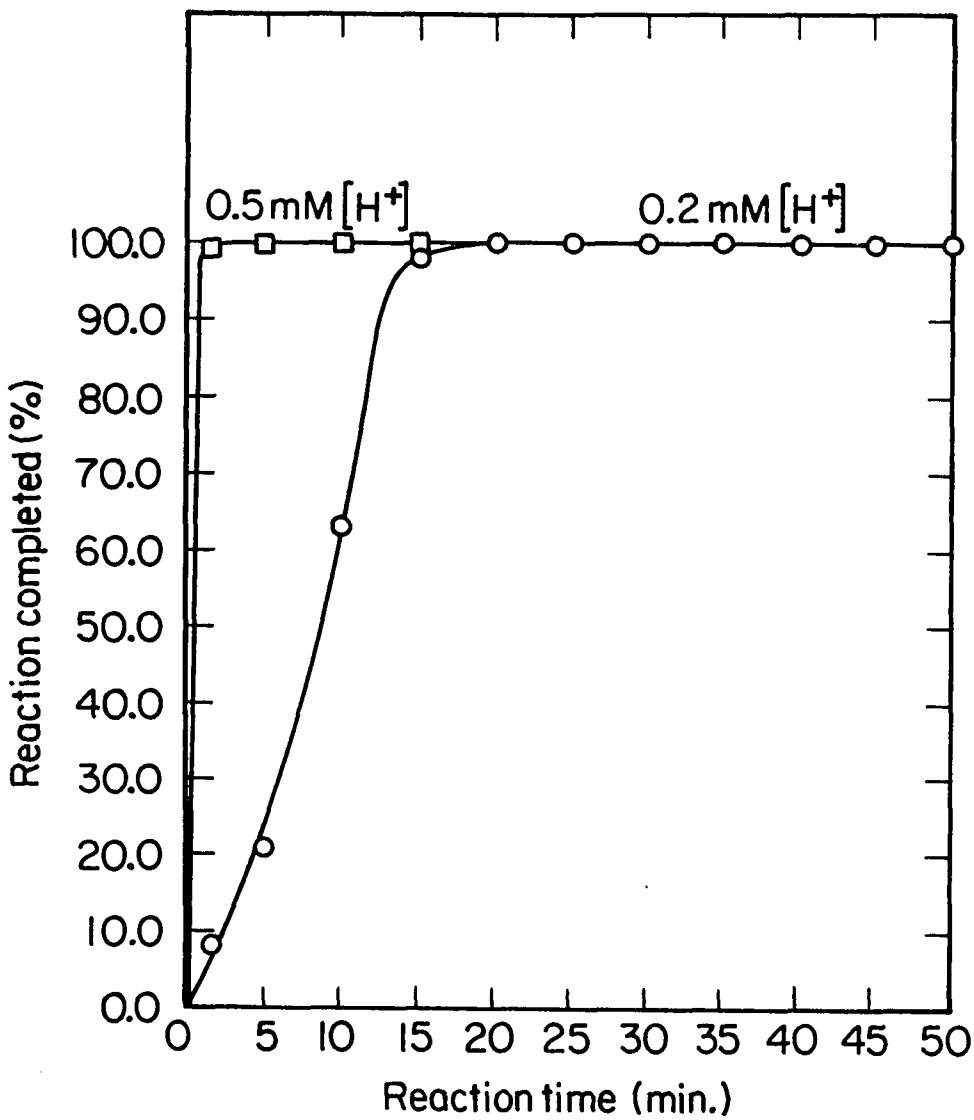


Figure 1. Percent reaction completed versus reaction time, obtained at different concentrations of methanesulfonic acid using TEOF as the reagent. Sample volume, 0.50 ml; reactant volume, 0.050 ml. Sample matrix, anhydrous DMF. Other conditions are given in the text

Table II. Minimum concentration of methanesulfonic acid required by various reagents for the reaction with water to be completed within 1 minute

Reagent	Minimum acid conc. required (mM)
2,2-Dimethoxypropane	5.0
Trimethylorthoformate	0.2
Trimethylorthoacetate	1.0
Triethylorthoformate	0.5
Triethylorthoacetate	1.0
Triethylorthopropionate	1.0

concentration of methanesulfonic acid added to the reactant solution must be at least 5.5 mM, considering the 11 fold dilution when 0.050 ml reactant solution and 0.50 ml sample is mixed. A slightly higher concentration of methanesulfonic acid (10 mM) was employed in actual sample analysis to ensure a rapid reaction rate. DMP required a higher minimum acid concentration than any of the ortho esters.

Amount of Reactant Solution

In the early experiments, a large excess of reactant solution (1.00 ml) was used for all the samples (0.50 ml) regardless of their water content. This corresponds to a water concentration of about 20%. It was found later that better accuracy and reproducibility could be obtained for samples with low water contents by reducing the volume of the reactant solution. Also, a lower limit of detection was obtained when a smaller amount of reactant solution was used. In this regard, it should be recalled that the water signal of a sample is obtained by subtracting the blank water signal of reactant solution from the total water signal of the reaction mixture. By using less reactant solution, the blank resulting from water in the reactant was reduced dramatically.

Use of 0.050 ml of reactant solution is recommended for samples expected to contain less than 1% water. If the water concentration of a sample is greater than 1%, which is indicated by the disappearance of the unspent TEOF reagent peak, a larger volume of reactant solution (e.g., 0.50 or 1.00 ml) is then necessary. Alternatively, the water content of a sample can be accurately determined by first employing a large excess of reactant solution and then using a smaller volume of reactant solution. These procedures are recommended to ensure good accuracy and reproducibility for the analysis of samples containing a

wide concentration range of water.

Chromatographic Conditions

Using a fixed flow rate (1.9 ml/min), GC conditions were determined so that isothermal elution of the excess reagent would be complete within 5 minutes. The oven temperature ranged from 40°C for TMOF to 110°C for TEOP; 80°C was used for TEOF.

Under the isothermal conditions employed, good resolution of the major components of reaction mixture was obtained. A typical chromatogram for determining water in a N,N-dimethylformamide (DMF) sample is given in Figure 2. The retention times were as follows: ethanol (product 1) = 1.09 min, ethyl formate (product 2) = 1.17 min, 3-methylpentane (internal standard) = 1.31 min, DMF (sample matrix) = 2.61 min, and TEOF (unspent reagent) = 4.14 min. Most samples can be analyzed in less than 5 minutes. A temperature program was used to rapidly elute any sample compound with a high boiling point.

Calibration Curve

Standard samples were prepared and analyzed, ranging from almost 0% water in anhydrous DMF to 100% water. Linear calibration plots were obtained over this entire dynamic range with correlation coefficient,

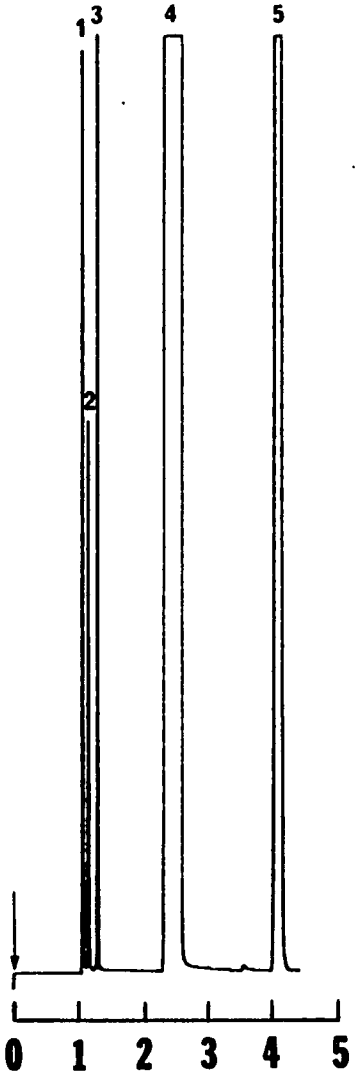


Figure 2. Chromatogram of a reaction mixture with 0.050 ml TEOF reaction solution and 0.50 ml DMF containing 0.187% H_2O. Peak assignment: 1, ethanol; 2, ethyl formate; 3, methylpentane; 4, DMF; 5, TEOF. Other conditions are given in the text

r = 0.99999 using the ethanol or ester peak, and r = 0.999 using the reagent peak.

Results were compared using DMP and TEOF reagents, catalyzed in both cases with methanesulfonic acid added to the reagent (Figure 3). The water concentration ranged from essentially 0.00% to 0.80 %. The calibration plots were linear for both reagents, but the slope of the TEOF plot was more than 2.6 times greater than that of DMP owing to a greater number of carbon atoms contained in the product of TEOF (ethanol). Negative responses were obtained with DMP below 0.004% water.

Effect of Acid Concentration, Type, and Sample Matrix

To study these effects, calibration plots were constructed under various conditions. A sample volume of 0.50 ml and a reactant volume of 0.050 ml was employed for all the calibration plots. Two calibration plots were obtained using reactant solutions containing 5.5 mM and 55 mM hydrochloric acid, respectively (Figure 4). The slopes of the two plots were essentially the same. This indicates that the concentration of an acid catalyst does not affect the sensitivity (slope of the calibration plot) provided a necessary minimum concentration of acid (5.5 mM in reactant solution, or 0.5 mM after dilution by sample matrix) is used.

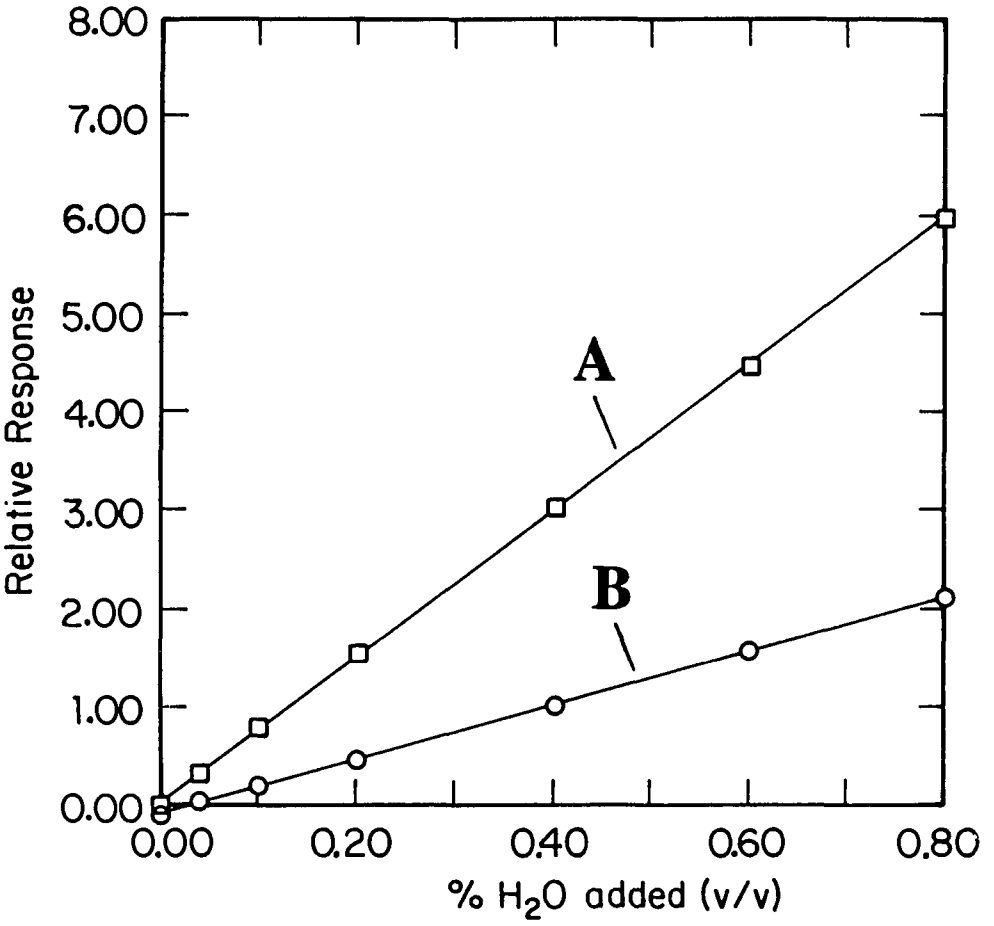


Figure 3. Calibration curves obtained using TEOF and DMP as the reagent. A, TEOF reagent; B, DMP reagent. Sample volume, 0.50 ml; reactant volume, 0.050 ml. Sample matrix, anhydrous cyclohexane. Other conditions are given in the text

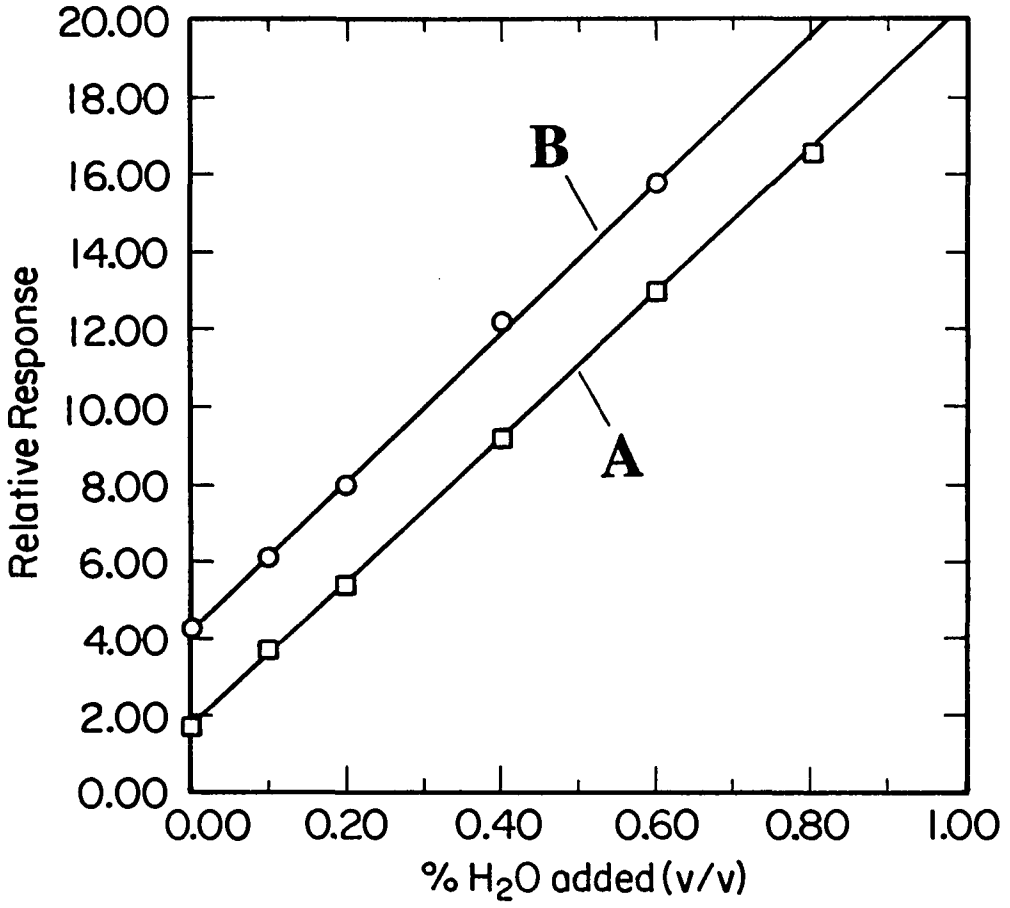


Figure 4. Calibration curves obtained with reactant solutions containing different concentrations of hydrochloric acid. A, 5.5 mM HCl; B, 55 mM HCl. Sample volume, 0.50 ml; reactant volume, 0.050 ml. Sample matrix, anhydrous DMF. Other conditions are given in the text

The different intercepts in Figure 4 resulted from the different amounts of water introduced with the different amounts of acid catalyst. Very similar slopes were also obtained using reactant solutions containing 5.5 mM and 55 mM methanesulfonic acid.

Different types of strong acids had essentially no effect on the sensitivity. Very similar slopes for the calibration plots were obtained using reactant solutions containing 5.0 mM methanesulfonic acid, hydrochloric acid, or 2.5 mM sulfuric acid (Figure 5).

Three distinct types of inert organic solvents (unreactive toward an ortho ester reagent) were tried to determine the effect of sample matrix. Calibration plots with essentially the same slope were obtained with ethyl acetate, dimethylformamide, or cyclohexane as the sample matrix (Figure 6). The different intercepts resulted from the different amounts of water originally present in the sample matrices.

This indicates that the sensitivity (slope of the calibration plot) for a given sample to reactant volume ratio (e.g., 0.50 ml/0.050 ml) can be determined by using only one sample matrix. Once the sensitivity has been established, the water content of other samples can be easily determined by dividing the corrected relative response by the sensitivity.

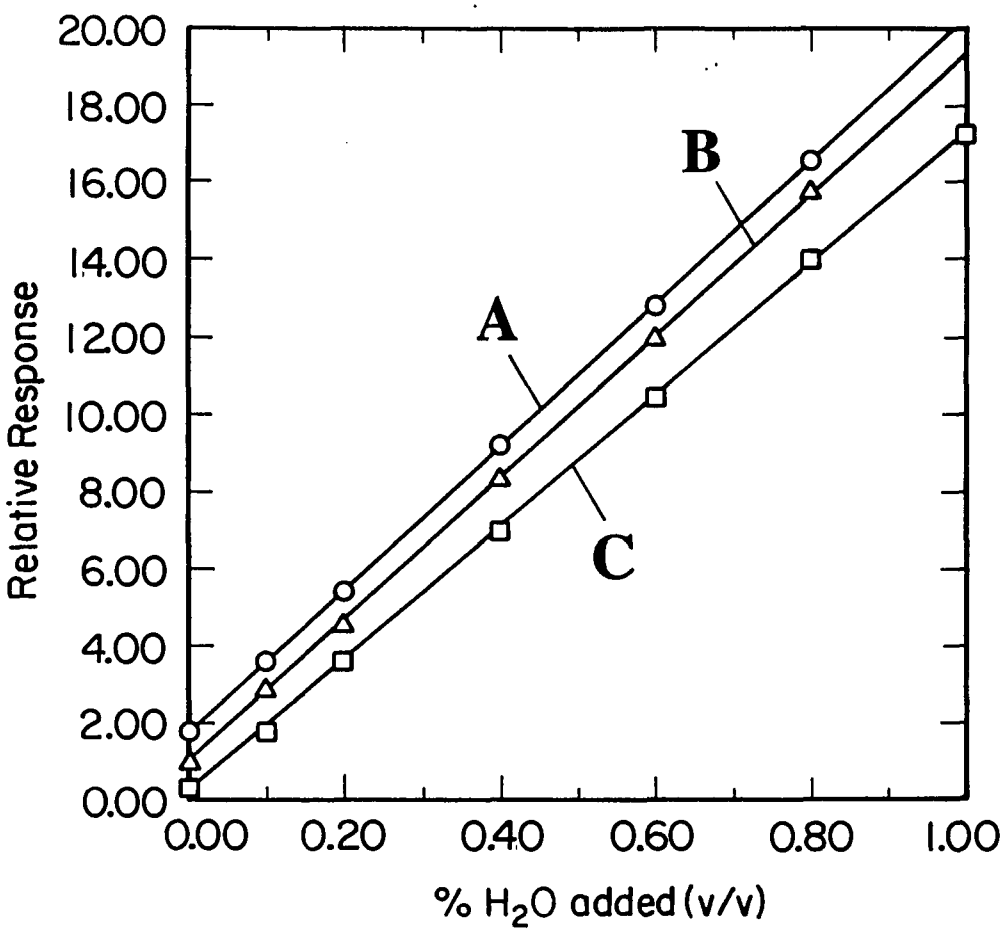


Figure 5. Calibration curves obtained with reactant solutions containing different types of acid catalyst. A, 5.0 mM hydrochloric acid; B, 5.0 mM methanesulfonic acid; C, 2.5 mM sulfuric acid. Other conditions are the same as given in Figure 4

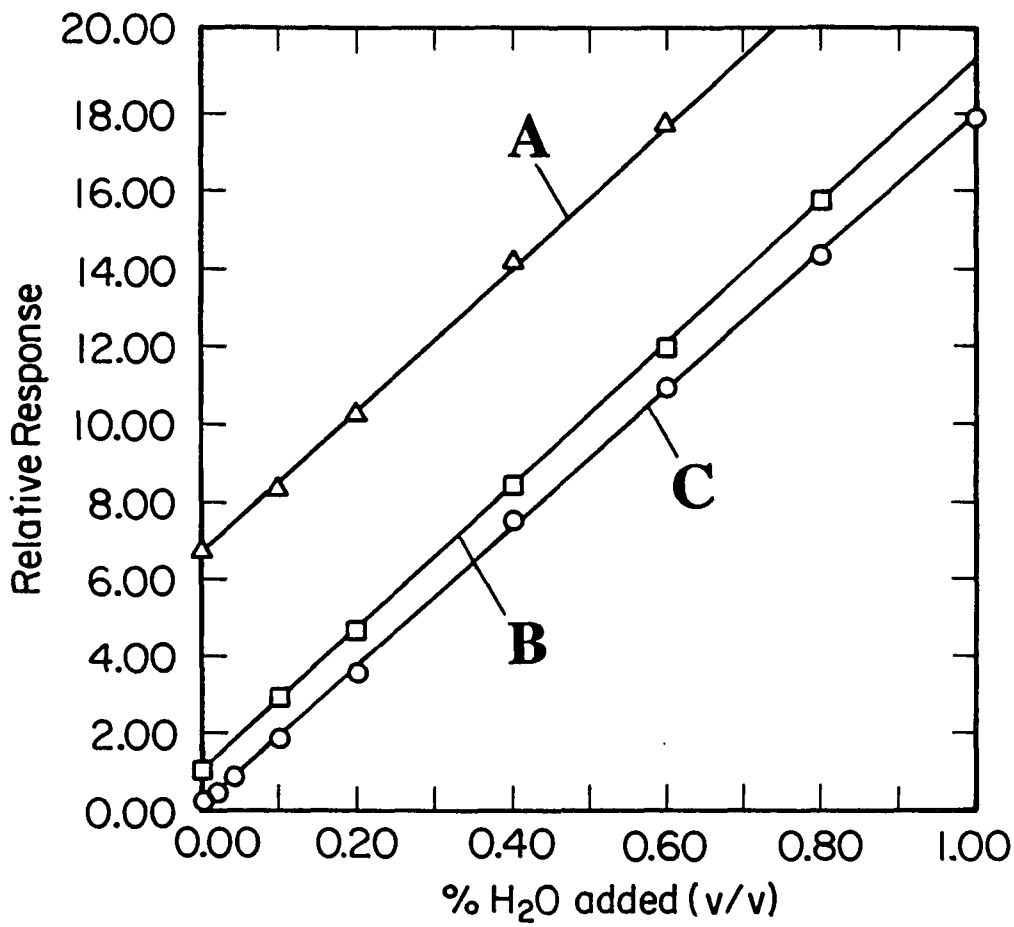


Figure 6. Calibration curves obtained with different sample matrices. A, ethylacetate; B, DMF; C, cyclohexane. Acid catalyst in reactant solution, 10 mM methanesulfonic acid. Other conditions are the same as given in Figure 4

Reproducibility, Limit of Detection

Three different samples were analyzed for water content six times each to determine the relative standard deviations. The data in Table III show that the relative standard deviation is larger for samples of very low water content. A substantial portion of the variation appears to come from the gas chromatographic step.

The lowest concentration of water actually determined was 0.00134% (13.4 ppm) in anhydrous cyclohexane. The limit of detection (S/N=3) in this case was estimated as 3 ppm. This was based on the standard deviation of 1 ppm for anhydrous cyclohexane. An even lower limit of detection should be possible by using splitless injection or a larger injection volume.

Accuracy of the Method

Various samples were analyzed both by the GC method and the standard Karl Fischer method (Table IV). The two methods showed good agreement for all the samples analyzed. The precision obtained for a particular sample by the GC method was similar to that by Karl Fischer method and was usually better than 5%.

Table III. Relative standard deviations determined for several samples with varying water concentrations

Sample	% H_2O found (v/v)[a] (n = 6)	Rel. Std. Dev.
Ethyl Ether	0.530 ± 0.005	0.94%
	(0.528 ± 0.001)	0.19%
Benzene	0.0156 ± 0.0004	2.6%
	(0.0152 ± 0.0002)	1.2%
Cyclohexane	0.00134 ± 0.00010	7.3%
	(0.00139 ± 0.00007)	5.0%

[a]Data without parenthesis corresponds to the result of six parallel analyses; data with parenthesis corresponds to the result of six repeated injections from the same sample vial

Table IV. Analysis of several samples using both GC method and Karl Fischer titration method

Sample	% H_2O found (v/v) (n = 3)	
	GC method	KF titration
Benzene	0.0156	0.0166
Cyclohexane	0.00134	0.00143
1,2-Dimethoxyethane	0.193	0.181
N,N-Dimethylformamide	0.504	0.499
Ethyl acetate	0.530	0.535
Nitromethane	0.190	0.202

Determination of Water in Various Samples

The percentage of water in a large variety of actual samples was determined both by the GC method and by a previously published liquid chromatographic method (5,6). The results are summarized in Table V. The difference between each individual analysis of the same sample was usually less than 5% for both methods. In most cases good agreement was obtained between the two methods. The recovery of an additional 0.50 mg of water added to the sample constituted another check on the accuracy of both methods. For solid samples the value in parentheses is the percentage of water if the compound has exactly the amount of water of hydration expressed by the formula.

The results in Table V show that all classes of compounds studied can be analyzed accurately for water except alcohols which are known to undergo exchange reaction with the ethoxy groups in the reagent. However, alcohols can be easily analyzed by the LC method (5). Aldehydes, ketones, and carboxylic acids also react with ortho esters and would be expected to interfere with the GC determination of water. The water content of amines (organic base), dimethylformamide, and dimethylsulfoxide can be determined by GC but not by the LC method (5).

Table V. Determination of water in various samples using both GC and LC methods

Sample	% water (v/v) (n = 2)		Recovery of 0.50 mg Water spike (mg)	
	GC method	LC method	GC method	LC method
HYDROCARBONS				
Decane	0.0071	0.0071	0.49	
Cyclohexane	0.0045	0.0043	0.51	
2-Ethyl-1-hexene	0.133	0.131	0.40	
Toluene	0.0198	0.0121	0.47	
HALOGENATED				
1,2-Dichloropropane	0.0310	0.0305	0.46	
1-Bromo-3-methylbutane	0.0109	0.0118	0.50	
Tetrachloroethylene	0.0050	0.0057	0.49	
ETHERS				
Tetrahydrofuran	0.0729	0.0722	0.48	0.51
1,2-Dimethoxyethane	0.160	0.159	0.53	0.49
1,2-Bis-(2-chloroethoxy)-ethane	0.108	0.105	0.48	
1,4-Dioxane	0.165	0.153	0.54	0.45
ALCOHOLS				
Isopentyl alcohol	0.111	0.390	0.10	0.51
Benzyl alcohol	0.692	1.64	0.12	0.50
Ethylene glycol	0.027	0.118		0.51
ESTERS				
Ethyl acetate	0.307	0.304	0.48	0.50
Dimethyl phthalate	0.198	0.234	0.55	0.51

Table V. (Continued)

Sample	% water (v/v) (n = 2)		Recovery of 0.50 mg Water spike (mg)
	GC method	LC method	GC method
MISC COMPOUNDS			
Dimethylsulfoxide	0.0831		0.52
N,N-Dimethylformamide	0.0469		0.51
Nitromethane	0.138	0.142	0.50
Benzonitrile	0.123	0.129	0.47
Carbon disulfide	0.0045	0.0037	0.48
PEROXIDES			
tert-Butyl peroxide	0.0377	0.0332	
2-Butanone peroxide	12.3	10.3	
Benzoyl peroxide	17.8	14.9	
ANHYDROUS SOLVENTS			
Decahydronaphthalene	0.0044	0.0026	0.52
m-Xylene	0.0105	0.0111	0.51
1,2-Dichloroethane	0.0072	0.0068	0.47
Butyl ether	0.0151	0.0142	0.48
1,3-Dioxolane	0.0440	0.0347	0.49
Anisole	0.0078	0.0064	0.51

Table V. (Continued)

Solid sample	% water (w/w) found (expected)	
	GC method	LC method
Cobalt chloride ($CoCl_2 \cdot 6H_2O$)	43.8 (45.6)	
Lithium perchlorate ($LiClO_4 \cdot 3H_2O$)	32.8 (30.0)	
Sodium tartrate-2-hydrate	15.6 (15.66 ± 0.05)[a]	15.8
Phloroglucinol dihydrate	22.8 (22.2)	
Lactose ($C_{12}H_{22}O_{11} \cdot H_2O$)	5.4 (5.1)	
Amoxycillin trihydrate	12.7 (12.6 - 13.2)[b]	13.1

[a]Obtained as water standard from Riedel-deHaën

[b]Provided by Beecham Parmaceuticals using various analytical methods

CONCLUSIONS

A simple, fast, and reliable GC method for the determination of water in a wide variety of samples has been developed. Reaction of water with triethylorthoformate is far more complete than with the DMP reagent used previously. The idea of using a liquid acid as the catalyst was successful. A complete determination of water, including the reaction step and the chromatographic separation requires only about five minutes. Linear calibration plot is obtained for water concentrations ranging from essentially 0.0% to 100%. Good sensitivity and low limit of detection is achieved with optimized conditions. More than 1000 injections were made throughout the entire work without any observable deterioration of the GC column. The current GC method is broad in scope and complements the previous LC method.

The GC method is fast and convenient, and it uses a smaller sample size than the Karl Fischer titration method. Other than a standard GC system, no special dedicated equipment is required. Similar reproducibilities were obtained for the two methods. Lower alcohols and carboxylic acids interfere with the GC method but not the Karl Fischer method. On the other hand, unsaturated organic compounds, mercaptans, and peroxides can be analyzed by the GC method but not the Karl Fischer

method. Other compounds such as aldehydes and Ketones interfere with both methods. However, these samples can be analyzed by the two-column method described in Section II.

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GENERAL SUMMARY

In this work, two liquid chromatographic and one gas chromatographic methods for the determination of water are developed.

In the first LC method, a unique spectrophotometric detection system is developed. Water in a large number of inert samples is determined quickly and accurately using only a short and small cation-exchange column in H^+ form. In the second LC method, a combination of a Li^+-form separation column and a H^+-from catalytic column is employed. Difficult samples which cannot be analyzed by either the single-column or the Karl Fischer method are analyzed quickly and accurately. In the GC method, an orthoester reagent is found to give more complete reaction with water than 2,2-dimethoxypropane which was used previously (6). Also, a liquid acid catalyst is found to give a faster reaction rate and a simpler analytical procedure than does the solid acid catalyst used in the previous method (6).

The LC methods and GC method complement each other and provide some advantages over the conventional Karl Fischer titration method, for instance, faster analytical speed, smaller sample size, better sensitivity, lower limit of detection, and fewer interferences.

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